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On Sensory Innervation of Larynx in Dog

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Much light has been thrown on the subject of the innervation of the mucous membrane of human larynx by the minute studies undertaken recently by Sasaki,[1] Matsumoto[2] and Momono,[3] while only a few cases of histological studies have been hitherto reported on the same subject in mammals. Moreover the greatest number of such studies were those carried out in the latter half of the last century (Lindemann 1869,[4] Boldyrew 1871,[5] Simanowsky 1883,[6] Retzius 1892,[7] Arnstein and Ploschko 1897[8]), all limited in the scope of the subjects and executed with the help of immature staining methods available in those days. These factors debarred them from getting at a number of meritorious informations. The author of this report took dogs as the object of the study, used the latest silver method and studied the views obtained in comparison with human specimens, to elucidate the biological meaning of the subject. In details, I adopted the following process: Materirals were fixed for a long time in 10% neutral formol, then cut into 40 μ transverse and longitudinal sections, and stained with the silver impregnation of Seto used in this laboratory. The series of many beautiful tissue preparations obtained thereby I subjected to a minute microscopic scrutiny.

Individual Observations

Histological picture of mucous membrane of larynx in dog:—

As clarified in the studies of the mucous membrane of human larynx by Sasaki,[1] Matsumoto[2] and others, the development or the state of distribution of nerves, especially sensory nerves, shows considerable difference as the histological piture differs, though the organ may be the same. This fact was also collaborated in my study on the canine larynx. So, I will preface my detailed description on the innervation with a word upon the histologial picture of the subject parts.

The fine structure of mucous membrane of larynx in dog shows no essential difference in comparison with that in man, but it is smaller and

simpler in scale. This fact is by itself enough to warrant assumption of weaker development of nerves in canine larynx than in human counterpart. Also the following points may be mentioned to show the histological difference in some extent between larynges of dogs and men. Tunica propria and tela submucosa are poorer in development by dogs, and the glandular and lymphatic tissues, especially the lymphatic nodes, formed therein, are too poorly developed to stand in comparison with the human specimen. The epithelium is composed, as is the case with man, of stratified flat epithelium and multi-lined ciliated epithelium. But their arrangement is somewhat different from that in man, the stratified flat epithelium lining the greater part of larynx, the ciliated epithelium appearing only at pars intercartilaginea of rima glottidis and pars inferior laryngis.

Upon nearer observation, the stratified flat epithelium found on the pharyngeal surface of epiglottis of dog is much lower than in man and contains small papillae, while that on the laryngeal side is almost utterly devoid of papillae and consists of very thin epithelium with a nearly smooth basal surface. This epithelium becomes further lower in height in pars intercartilaginea of rima glottidis and ventriculus laryngis, in parts only three layers of epithelial cells being observable.

Nerve plexus in mucous membrane of larynx:—

The nerve elemtnts supplying the mucous membrane of canine larynx are represented by n. laryngicus cranialis of n. vagus and the sympathetic nerves originating from truncus sympathicus cervicalis. The former is composed of thick sensory nerve fibres, containing minute parasympathetic fibres, which, penetrating the membrana hyothyreoidea, run to the outer side of the basis of epiglottis and disperse into a number of nerve bundles there. It is not hard to assume that they receive anastomotic rami from the sympathetic nerves in their course, as is known in human cases.

Thus, the nerve elements for the mucous membrane of larynx are composed of thick medullated sensory and fine non-medullated vegetative fibres. Now, the nerve elements to the epiglottis mostly head for the laryngeal side thereof, only a very few of them running to the pharyngeal side. The nerve branches to the laryngeal face run along the outer side of epiglottis, and reach the laryngeal side through the numerous pores in cartilago epiglottidis to form plexus submucosus mainly spreading out in flat planes. Minute rami are derived from this plexus, which penetrate into the tunica propria directly beneath the epithelium, to form plexus tunicae propriae. The development of these plexus is especially marked in the central part of the laryngeal side.

The nerves to the pharyngeal side of epiglottis are, on the contrary, represented by small quantity of the nerve elements directly diverted from

the outer basal part of epiglottis. Plexus formation in submucosa and propria is also found here, but in incomparably poorer development than on the laryngeal side.

As described above, the plexus formation in the canine epiglottis is nothing essentially different from that in man, but the development is in far lower stage than in adult human beings (Sasaki[1]), and is more similar to that in tenth month human embryo (Momono[3]).

The nerve fibres to the mucous membrane of larynx except epiglottis are somewhat poorer than those to epiglottis. They penetrate into the mucous membrane of ventriculus laryngis from plica ventricularis, thence through the glottal part to pars inferior laryngis, to form in all these parts plexus submucosus and plexus laminae propriae in the same manner as in epiglottis. These plexus rarely contain multipolar nerve cells. The development is rather good in plica ventricularis and pars intercartilaginea rimae glottidis, very poor in pars intermembranacea of the same, and comparatively weak in ventriculus laryngis and pars inferior laryngis.

Sensory innervation of mucous membrane of epiglottis:—

Studies were undertaken by Lindemann,[4] Retzius[7] and Arnstein and Ploschko[8] on the subject in connection with larynx of mammals, but the results attained have now only historical meanings, being almost useless for reference. In my study I have found that, similar to human counterparts (Sasaki),[1] epiglottis is the part most richly provided with sensory fibres in the laryngeal mucous membrane. However, the development of sensory fibres, especially their terminations, is far poorer in dog than in man.

Firstly, the sensory endings connected with the Hering's falling reflex of blood pressure are in human adult represented by two types of terminations with peculiar structure, which were found in sinus caroticus (Sunder-Plassmann,[9] de Castro,[10] Ohtomo[11]), in arcus aortae and atria cordis (Seto[12]), in trachea (Sasaki[1]) and bronchi (Sunder-Plassmann[9]). Especially, in the adult human epiglottis, very typical specimen of such terminations were found by Sasaki.[1] Then, in epiglottis in dog, I made thorough microscopic search, but I failed in discovering the Type I termination, that is, any of the complex branched sensory terminations with neurofibrillar terminal plates (Seto[12]) as the most conspicuous characteristic.

On the contrary, the Type II endings constructed far more simply, were found rather frequently, especially in the perichondrium of the laryngeal side of epiglottis, in the shape of branched sensory terminations arranged irregularly over a comparatively wide area. In the territory of these terminations, small blood vessels, especially small arteries are found accompanying the nerve fibres in almost all cases, which condition

Fig. 1

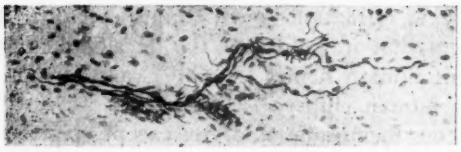

Fig. 2

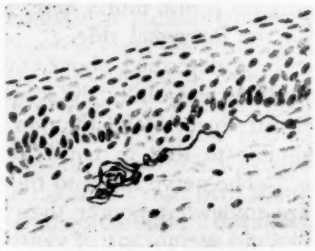

Fig. 1. Termination Type II related to blood pressure falling reflex found in perichondrium of the laryngeal side of epiglottis in dog. a small artery. Seto's impregnation. X 320, reduced to ½.

Fig. 2. Simple glomerular termination found in tunica propria directly beneath the epithelium of the laryngeal side of epiglottis in dog. Same staining. X 400, reduced to ½.

Fig. 3

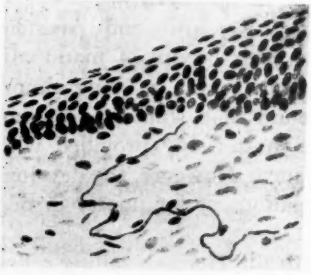

Fig. 4

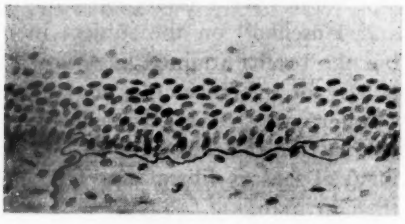

Fig. 3. Unbranched termination found in tunica propria of the laryngeal side of epiglottis in dog. Same staining. X 400, reduced to 2/3.

Fig. 4. Simple branched termination found directly under the epithelium of the laryngeal side of epiglottis in dog. Same staining. X 320, reduced to 2/3.

is one of the characteristics of the terminations in question. The branches of such terminal fibres mostly end sharply (Fig. 1).

Thus the sensory terminations for blood pressure falling reflex may exist also in epiglottis of mammals, but the fact that they are always represented by the simpler terminations of Type II seems to suggest that the occurrence of complex terminations showing extremely complicated structure as in Type I is very rare in non-human animals, which situation is also apparently proved by the following observation.

In the subepithelial connective tissue of adult human epiglottis have been located a large number of corpusclar terminations, especially glomerular terminations, either capsulated or non-capsulated (Sasaki[1]), but in the canine epiglottis no such complex formation of corpusclar termi-

Fig. 5

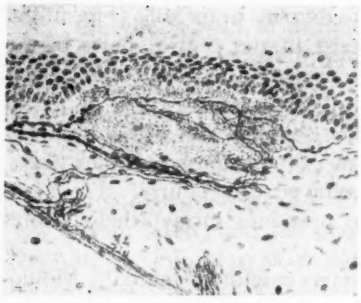

Fig. 6

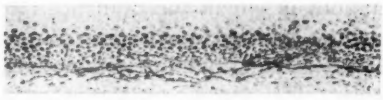

Fig. 5. Complex branched termination formed in a lymphocytes gathering under the epithelium of the laryngeal side of epiglottis in dog. Same staining. X 200, reduced to ½.

Fig. 6. Complex indifinite branched termination formed under the epithelium of laryngeal side of epiglottis in dog. Same staining. X 320, reduced to ½.

Fig. 7

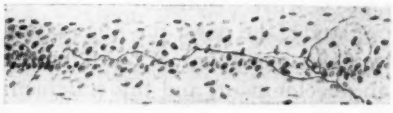

Fig. 8

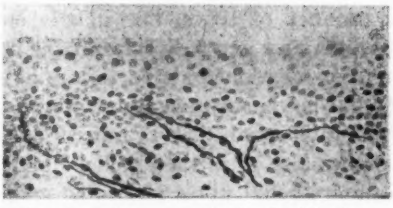

Fig. 7. Fine branched intraepithelial fibres found on the laryngeal side of epiglottis in dog. Same staining. X 320, reduced to ½.

Fig. 8. Thick intraepithelial fibres found on the laryngeal side of epiglottis in dog. Same staining. X 320, reduced to ½.

nations was found anywhere, only a few very simple non-capsulated glomerular terminations being discovered directly under the epithelium (Fig. 2). If we recollect that by man, such glomerular terminations, though in somewhat lower development, are already present in tenth month human embryo, the development of sensory terminations in canine larynx may be called infantile indeed.

As we can easily understand from the above description, the majority of sensory terminations in epiglottis in dog is represented by unbranched endings of simplest structure (Fig. 3) and simple branched endings (Fig. 4). In these terminations, the nerve fibres, having lost the myelin, end sharply or often bluntly in tunica propria directly beneath the epithelium without any branching (unbranched terminations) or divided into 2

or 3 branches (branched terminations). Sometimes, however, branched terminations are found showing relatively complex branching (Fig. 5 and Fig. 6). Especially, such terminations exist in the central parts of the laryngeal face of epiglottis, being often represented as plexus-like terminations in which the branches show bush-like arrangement, or as terminations ending in non-descript from. The sensory fibres and branches forming these complex terminations are generally composed of thick fibres, which show specific change in size and specific winding course. Frequently, they penetrate into the epithelium to pass into intraepithelial fibres described in the following.

In canine epiglottis, as well as in human epiglottis (Sasaki[1], Momono[3]), sensory fibres are observed entering the epithelium and forming intraepithelial fibres. Their development is somewhat weaker than in human adult, but it may be described as rather well-developed, in particular, remarkably so at the central parts of the laryngeal side. On the pharyngeal side, however, their existence is hardly observable. In human epiglottis, intraepithelial fibres are better developed in adult (Sasaki[1]) than in tenth month embryo (Momono[3]), and Sasaki classed them into a number of types, but in the embryonal stage, the terminations are simpler and generally classifiable into three types (Momono[3]).

As mentioned above, by my object of study, the dogs, the innervation of the laryngeal mucous membrane resembles more that of tenth month human embryo, than of human adult, in development, and this statement applies also to intraepithelial fibres. That is to say, the development of intraepithelial fibres in canine epiglottis is in general as much advanced as that of tenth month embryo (Momono[3]), and their terminations may be fitly divided into the three following types.

The Type I is represented by fine fibres with smooth surface (Fig. 7), the Type II by thicker fibres with frequent change of size (Fig. 8), the latter surpassing the former in quantity, while the Type III represents the intermediary form of the two types above. These types may originate in all kinds of terminations formed in propria as described above and are classifiable into unbranched and branched terminations, the latter again into simple and complex ones. The course of these fibres in the epithelium is very irregular, i.e. they run sometimes perpendicularly, sometimes bliquely, and sometimes in planes parallel to the basal layer and terminate in variable heights of the epithelium. Some terminal branches revert into the propria, some of them emerging again into the epithelium. The intraepithelial fibres, as is usual for such fibres, take their course between the epithelial cells as well as right through the cell bodies and terminate sharply or bluntly, but sometimes in small nodes, which are seen usually by thick fibres.

Sensory terminations in laryngeal mucous membrane except epiglottis:—

On the subject, Matsumoto[2] and Momono[3] of this laboratory reported on their studies in full on human adult and tenth month human embryo respectively. They clarified the distribution of sensory nerves and based their morphological classification of termination types thereupon. My observations on the canine larynx are in general harmony with their observations, but in development, the innervation is more in resemblance with that of tenth month embryo, standing far back to that of human adult, and the general conclusion was that the sensory innervation of canine larynx is much poorer in development than the case is with human beings. Let me state my observations in detail hereunder.

Also in the mucous membrane of canine larynx except the epiglottis the development of sensory fibres and their terminations is in proportion to the development of plexus submucosus and plexus tunicae propriae. The development is at the best in plica ventricularis, second only to the epiglottis, poorer in pars intercartilaginea rimae glottidis, and poorest in pars intermembranacea rimae glottidis. The mucous membrane of pars inferior laryngis and ventriculus laryngis is also rather poorly provided with sensory fibres.

Upon the sensory terminations related with Hering's blood pressure falling reflex in the mucous membrane of larynx except epiglottis, Matsumoto discovered in human adult the terminations of the Type I in plica ventricularis and pars intercartilaginea rimae glottidis, and of the Type II in the perichondrium of cartilago arytaenoides and in lig. vocale respectively, and Momono[3] found in 10th month embryo the germs o both the types at the same places. In my study on canine larynx, I could not find existence of Type I terminations with neurofibrillar plates anywhere, as was observed in the case of epiglottis. On the contrary, Type II terminations are not rare in the extraepiglottid area, especially in perichondrium of cartilago arytaenoides as well as inside of lig. vocale. They consist of one or two thick stem fibres passing into comparatively simple branched terminations. The rami often show change in size and peculiar winding course, show irregular running arrangement in a wide terminal territory and are accompanied by small arteries (Fig. 9). These terminations are somewhat smaller in size than those found in epiglottis described above, and are simpler than the human counterparts.

Concerning the corpusclar terminations in the extraepiglottid area of larynx Matsumoto found glomerular terminations in the subepithelial tissue of plica ventricularis and pars intercartilaginea of human adult. Momono also found infantile type of glomerular terminations in the same places of tenth month human embryo. I found some comparatively

simple, non-capsulated glomerular terminations in the submucosa of plica ventricularis in dog (Fig. 10), but these are very few in number, and none could be found in lower parts, such as ventriculus laryngis, glottis and pars inferior laryngis.

Fig. 9

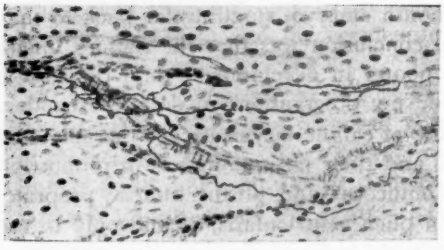

Fig. 10

Fig. 9. Termination Type II related to blood pressure falling reflex found beneath lig. vocale in dog. Same staining. X 320, reduced to $\frac{1}{2}$.

Fig. 10. Simple glomerular termination formed in the subepithelial tissue of plica ventricularis in dog. Same staining. X 400, reduced to $\frac{1}{2}$.

Thus, the major part of sensory terminations found in extraepiglottis area of larynx in dog is also represented by unbranched and branched terminations formed in subepithelial tissue, showing a development behind that in human adult. Locally, plica ventricularis is best provided, while pars intermembranacea rimae glottidis is the least favored in this respect.

The unbranched terminations are of the simplest type composed of medullated fibres losing their myelin and ending sharply or bluntly without branching, as described above in epiglottis. By branched terminations (Fig. 11) the medullated fibres, after losing their myelin, divide into 2 or 3, or sometimes more numerous, branches, which run the winding course peculiar to sensory fibres, to end sharply or bluntly. Some of these terminations are more complicated in formation and are represented as arborized or bush-like terminations according to the arrangement of branches. These complex terminations are simpler than those in laryngeal face of epiglottis and smaller in number.

Intraepithelial fibres exist also in extraepiglottid area of larynx (Fig. 12 and Fig. 13). The development of these fibres is also proportional to the development of nerve plexus in tunica propria, that is, considerably good in plica ventricularis and pars intercartilaginea, poor in ventriculus

Fig. 11

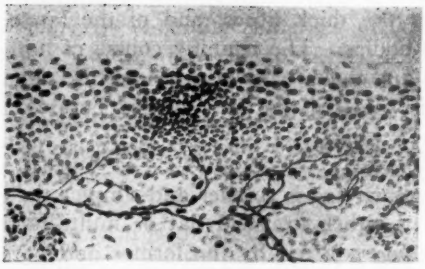

Fig. 12

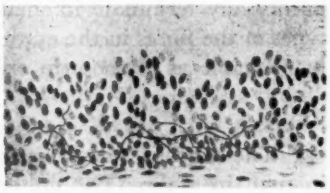

Fig. 11. Branched termination formed under the epithelium of plica ventricularis in dog. Same staining. X 320, reduced to ½.

Fig. 12. Intraepithelial fibres found in the multi-lined ciliar epithelium of pars intercartilaginea rimae glottidis in dog. Same staining. X 500, reduced to 2/3.

Fig. 13

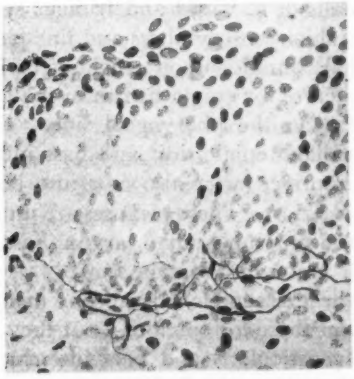

Fig. 13. Thick branched intraepithelial fibres formed in the basal layer of epithelium of plica ventricularis in dog. Some staining. X 400, reduced to ½.

laryngis and pars inferior laryngis, and poorest or nearly non-existent in pars intermembranacea. The intraepithelial fibres here are generally poorer in development than those in epiglottis, being more sporadic in distribution and simpler in structure. This state resembles closely the case of tenth month human embryo.

These intraepithelial fibres, similar to those in epiglottis, are classified into two types—the one originating in fine fibres with smooth surface and the other in comparatively thick fibres showing conspicuous change in size. In human specimens (Matsumoto[2] and Momono[1]), these fibres are mainly represented by thick fibres showing change in size, and especially in adult (Matsumoto[2]), the terminal fibres end in knob- or ring-

like formations. By dog the proportion is reversed, the fine fibres with smooth surface predominating over the thick fibres, and of the latter, hardly any terminate in small end-bodies. The running course of both types of the fibres in the epithelium and their terminal mode are the same in principle as of those in epiglottis, and will not be repeated here.

Summary

The mucous membrane of larynx in dog is smaller and simpler than in man in scale. The epithelium is either stratified flat or multi-lined ciliated, the major part of larynx being covered by the former and pars intercartilaginea and pars inferior laryngis only being surfaced by the latter type of epithelium. The height of epithelium is lower than that of man. These facts suggest the lower stage of development of nerves, especially sensory nerves in this part, in dog than in man.

The nerves supplyint the laryngeal mucous membrane are originated in n. laryngicus cranialis of n. vagus and truncus sympathicus cervicalis. The former consists of thick sensory fibres and fine parasympathetic fibres, which divide into small bundles upon reaching to basis of epiglottis.

The majority of nerves for epiglottis run over the laryngeal side, only a small minority going to the pharyngeal side. The former penetrate the small pores of cartilago epiglottidis and pass into plexus submucosus and plexus tunicae propriae, latter also pass into both plexus, but their development is very inferior. These plexuses are much more backward in development than those in adult human larynx (Sasaki), resembling more those in tenth month embryo (Momono).

The nerves for the extraepiglottid mucous membrane of larynx are less well-developed than those for epiglottis, but they may be called rather strong only in plica ventricularis and pars intercartilaginea, being poor in ventriculus laryngis and pars inferior laryngis and extremely poor in pars intermembranacea.

As in man, the epiglottis is best provided with sensory fibres in the whole area of larynx. Yet their development is far below that of human larynx. Firstly, in man, both the Types I and II of sensory terminations related with Hering's blood pressure falling reflex are formed conspicuously, in dog, only the simpler Type II is observed. Sencondly, in man, the existence of complex corpursclar terminations is clarified, whereas in dog, only a few very simple non-capsulated glomerular terminations are observed. This situation is much in similarity with the larynx in tenth month human embryo.

Thus, the sensory terminations in the mucous membrane of canine larynx are mostly represented by simplest unbranched and simple branched terminations. However, more complex branched terminations, such as

plexus-like terminations are also observed, though in a small quantity. They are generally composed of thick fibres showing peculiar change in size and winding course.

Besides, intraepithelial fibres are demonstrated. They are lower in development than in human adult, but very remarkable on the laryngeal side. Their terminal formation, however, is more similar to those in tenth month human embryo than those in adult human, and classifiable into three types. The Type I consists of fine fibres, the Type II of thick fibres with frequent change in size and the Type III represents an intermediary form of the two former. All of them are again divisible into unbranched and branched terminations. These fibres run in the epithelium very irregularly, to end finally sharply or bluntly.

Sensory terminations in extraepiglottid area of larynx in dog are also much poorer developed than in adult man, resembling more those of tenth month human embryo. The development of sensory fibres is also here parallel to the development of plexus in subepithelial tissue, being at best in plica ventricularis, next in pars intercartilaginea, poor in ventriculus laryngis and pars inferior laryngis and poorest in pars intermembrananacea.

There are two types of sensory terminations related with Hering's blood pressure falling reflex in man, but only Type II terminations are observable in dog, which are formed simpler than in human adult. Corpusclar terminations are found in many places in man, while in dog, only very infantile non-capsulated glomerular terminations are found in plica ventricularis, and in no other parts.

Thus the sensory terminations are also here represented, as in the epiglottis, by unbranched and branched terminations in main, their development being in proportion to the development of plexus in subepithelial tissue. Accordingly, the development is highest in plica ventricularis, and poorest in pars intermembranacea. Some comparatively complex branched terminations are also found, but they are all simpler in structure and smaller in quantity than those in epiglottis.

Intraepithelial fibres are also well-developed in plica ventricularis and pars intercartilaginea, poorer in ventriculus laryngis and pars inferior laryngis, and poorest in pars intermembranancea. They are also much worse-developed than in epiglottis, resembling those in tenth month human embryo, and are mainly represented by fine fibres and partially by thick fibres.

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On Innervation of Taste-Buds in Larynx in Dog

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Since Verson (1868) discovered the existence of taste-bud-like organs on the laryngeal face of epiglottis, many subsequent studies confirmed the existence. However, opinion was divided on their physiological meaning, that is, on the problem whether they were true taste sense organs as the taste-buds on the dorsum linguae. For example, Simanowsky[1] examined the nerves for the so-called goblet organs in larynx in man and mammals histologically, and came to the supposition that they were related with pressure or tactile sense rather than the sense of taste. Michelson (1891[2]), however, made it clear that taste sense organs exist also on the laryngeal side of epiglottis from the results of his experiment on human larynx. This fact is beyond doubt from the histological structure of the organs.

Recently, Sasaki[3] of this laboratory conducted a very minute histological research on the innervation of the taste-buds found in larynx of human adults and arrived at many new observations. I made a similar study, with stress upon the sensory innervation, on canine larynx, in succession to the study on the nerve supply of canine larynx,[4] utlizing the same preparations stained with Seto's silver method, as in my previous study. I will hereunder describe my views in comparison with those obtained by Sasaki in his study on human larynx.

As to the distribution of taste-buds in canine larynx, they were found most abundant on the laryngeal side of epiglottis, especially in its upper parts, next in plica ventricularis and on the innerside of cartilago arytaenoides, and in a number also in the glottid part and pars intercartilaginea rimae glottidis lined by the stratified flat epithelium. The above is in conformance with the description on the subject by Davis[5], and is not much different from what has been reported on the human larynx (Sasaki[3]).

The development of the nerve elements to such taste-buds is lower in dog than in man. They take their origin in the subepithelial pleuxs

and penetrate close to the base of the taste-buds, some ending in simple glomerular terminations or plexus-like terminations, but in most cases ending in unbranched or simple branched terminations. In rare cases, however, they penetrate further into the taste-buds or the epithelium around them, to end as intra- and extragemmal fibres.

For example, Fig. 1 shows the terminal state of taste nerve fibres to a taste-bud found in plica ventricularis. One or two thick sensory fibres coming from the subepithelial plexus reach the papilla under the taste-bud, showing peculiar change in size and running a winding course, and they finally pass into a simple glomerular termination consisting of thick fibres and thin fibres, a part of which further penetrating into the basis of the taste-bud, to terminate as intragemmal fibres.

Fig. 2 shows the innervation of taste-buds found on the inside of cartilago arytaenoides. Thick fibres and thin fibres terminate in the tunica propria directly under the taste-buds, showing comparatively complex plexus, from which appear intra- and extragemmal fibres ending sharply or bluntly. Figs. 3 and 4 show the sensory terminations for taste-buds found on the laryngeal side of epiglottis and in pars intercartilaginea rimae glottidis respectively. The nerve elements are from a very limited number of thick sensory fibres, which end either just beneath or in the taste-buds as intragemmal fibres, sharply or bluntly, without showing any noteworthy glomerular or plexus-like formation under the taste-buds. There is seen in Fig. 5 a simple plexus-like termination composed of fine fibres, found directly under a taste-bud in the glottid part. A few intragemmal fibres are observable going out from this termination.

As clarified in the above described figures, the nerve fibres supplying the taste-buds are classifiable into thick fibres and fine fibres, as the case is also with human larynx (Sasaki[3]). In canine larynx, however, unlike the human couterpart, the former predominates vastly, and fibres belonging to the finer type are very few. Indisagreement with the opinion of Sasaki[3], I presume the fine fibres also to be of sensory nature, and not vegetative fibres, because, especially as shown in Fig. 1, the fine fibres are derived from the thick fibres by branching, in the most part.

The taste-buds in canine larynx may be classified into the four types as follows, according to the mode of sensory innervation: 1. The taste-buds best provided with sensory nerves, being supplied by both the thick and fine fibres (Figs. 1 and 2); 2. These provided with thick fibres only (Figs. 3 and 4); 3. Those provided with fine fibres only (Fig. 5) and 4. Those not supplied at all, that is, the most poorly innervated type.

This classification is in harmony with that by Sasaki of human laryngeal taste-buds. However, by human larynx, the laryngeal side of epiglottis is provided with Types I and II of the above taste-buds, and is

Fig. 1

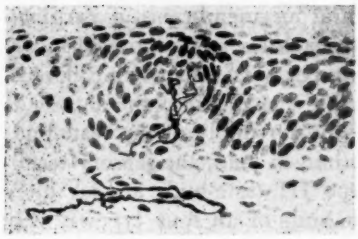

Fig. 2

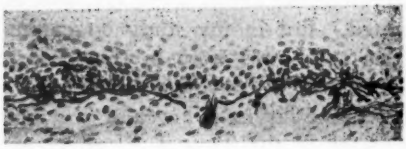

Fig. 1. A simple glomerular termination to a taste-bud found in plica ventricularis in dog. Seto's impregnation. X 500, reduced to 2/3.

Fig. 2. Plexus-like terminations composed of thick fibres formed under two taste-buds on the inside of cartilago arytaenoides in dog. Intra- as well as extragemmal fibres are discernible. Same staining. X 320, reduced to $\frac{1}{2}$.

Fig. 3

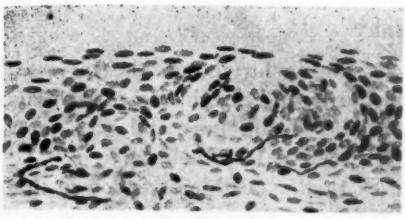

Fig. 4

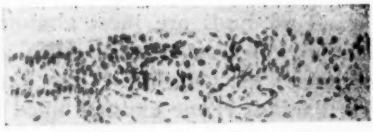

Fig. 3. Simple branched termination composed of thick fibres formed under a taste-bud found on the laryngeal side of epiglottis in dog. Same staining. X 500, reduced to $\frac{1}{2}$.

Fig. 4. Unbranched and simple branched terminations of thick fibres formed under two taste-buds existing only in the stratified flat epithelium of pars intercartilaginea rimae glottidis in dog. Epithelia of both sides consist of multi-lined cilieted epithelium. Same staining. X 320, reduced to $\frac{1}{2}$.

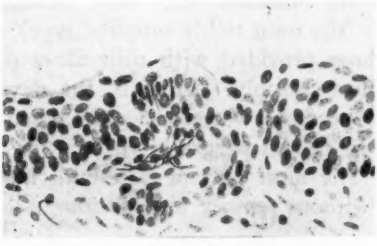

Fig. 5. Plexus-like termination composed of fine fibres formed under a taste-bud in glottid part in dog. Same staining. X 500, reduced to $\frac{1}{2}$.

richly supplied by sensory fibres, while the taste-buds on the glottid parts are of Types II and IV, those in the mucous membrane of cartilago arytaenoides are of Type III, that is, these parts are none too well innervated. But in my study on canine larynx, it was somewhat different. The taste-buds were in the most part of Type IV, i.e., the tate-buds lacked almost any sensory supply. The sensory supply of the taste-buds in canine larynx is lower in development than in man, the sensory terminations to the Type I, Type II and Type III taste-buds are far more simple, and the arrangement of the taste-buds of all four types is very disorderly. In man, according to Sasaki, the taste-buds are more imperfectly or degeneratively innervated as we go from the cranial to the caudal part, but in dog, this is not necessarily the case, some sensory terminations found in plica ventricularis (Fig. 1) and the inside of cartilago arytaenoides (Fig. 2) being in more complex formation than those in the laryngeal side of epiglottis. Even in the glottid part some considerably complex sensory terminations can be found.

Summary

Taste-buds are most abundant in canine larynx on the laryngeal side of epiglottis, next in plica ventricularis and the inside of cartilago arytaenoides. Some are also found in the glottid part and pars intercartilaginea rimae glottidis lined by stratified flat epithelium.

The development of sensory nerves to the taste-buds is lower than in man. The sensory fibres originate in the subepithelial plexus, and most of them pass into unbranched or simple branched terminations, though some are found ending in simple glomerular or plexus-like terminations directly under the taste-buds. In rare cases, fibres are seen further penetrating into the taste-buds, to form intra- and extragemmal fibres. These sensory fibres are composed in most part of thick fibres, and a smaller number of fine fibres, the latter as well as the former being probably of sensory nature.

The taste-buds are classified into those provided with both thick fibres and thin fibres (the most richly supplied type), those provided with only thick fibres, those provided with thin fibres only, and those that are lacking in all nerve supply. In man, the development of sensory nerves to taste-buds becomes worse as we go down from the laryngeal side of epiglottis to the caudal part of larynx, but in dog, this innervation is very disorderly. The most part of taste-buds in canine larynx is represented by those without sensory innervation.

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Constitution and Partial Structure of Group Lipoids*

By

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Group lipoids, prepared from human livers (Group A) and hog lungs in practically pure state, were saponified with 0.2 *N* NaOH. From each of them were obtained four fractions. Fr. I was a higher aliphatic alcohol; Fr. II contained palmitic acid and Fr. III several amino acids and α-glycerophosphoric acid; Fr. IV was a partial group lipoid, that proved homogeneous after electrophoresis. On the final fraction of the group lipoids further studies were made with following results: a) The group-active fragment of the human liver group lipoid consisted of 2 molecules of α-glycerophosphoric acid, 1~2 molecules of ethanolamine (differing according to the preparation), 2 molecules of glutamic acid, 1 molecule each of glycine and serine, 4 molecules of galactose, 2 molecules each of mannose and chondrosamine, and a few fatty acids, and that of the hog lung group lipoid of 4 molecules of α-glycerophosphoric acid, 1 molecule each of aspartic acid and ethanolamine, 2 molecules each of serine and glutamic acid, 4 molecules of galactose, 1 molecule each of glucosamine and chondrosamine, and a water-insoluble matter. b) i) The fragment of the human liver group lipoid was studied with DNFB, and glycine, one of the molecules of ethanolamine and the both chondrosamine molecules were found to contain a free amino group. ii) The same material gave more vivid ninhydrin reaction at pH's from 3 to 5 than at pH 7 when exposed preliminarily to the respective pH's at 50°C, and became to retain more of cupric ion or azocarmine in retention analysis when heated in advance with a dilute acetic acid of pH 1~2. It reduced Fehling solution but not a $HgCl_2$ solution of pH 7.5~8.0. The findings suggested the presence of a N-glycoside linkage in the molecule, so it was heated with 1 N acetic acid after once pairing with DNFB and then again treated with DNFB followed by HCl-hydrolysis. The DNP derivative of glutamic acid was detected among the acid-hydrolysis products. Therefore, this amino acid is suspected to partake in forming the N-glycoside link.

* Details were read before the 25th General Meeting of the Japanese Biochemical Society, Tokyo, April 26-28, 1953.

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On Innervation of Mucous Membrane of Larynx in Canine Fetus

By

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(Received for publication, December 29, 1952)

Very few histological studies on the innervation of mucous membrane in larynx have been reported, and what is more, these studies were done with the help of immature staining methods hitherto available, minimizing the merit of such studies, but recently, Sasaki[1] and Matsumoto[2] of this laboratory undertook minute studies on the innervation of larynx in human adult, and Momono[3] on that in human embryo, elucidating many problems on the subject.

In the previous report of Koizumi,[4] he has announced the results of his study on the innervation of mucous membrane of larynx in adult dog, especially of sensory nerves, in comparison with that of human larynx. In sequence thereto, we undertook a study on the same subject in connection with canine fetus, with a view to a genetical and anatomical research in comparison with human embryo. On this subject, there has been only a report of study by Fusari (1894[5]), as far our information goes, and his results gained by means of the immature staining method available at the time were far from meriting anything in the way of thoroughness.

We took the material of our study from two canine embryos of full months, and fixing them in 10% neutral formol for a long time, cut them into 40μ frozen sections, which were stained with Seto's impregnation. The series of beautiful preparations obtained we subjected to thoroughgoing microscopic scrutiny and arrived at the observations stated hereunder.

Individual Views

To give a general histological description of larynx of canine fetus, the development of the glandular and lymphatic tissues in the subepithelial connective tissue is far below that of grown-up dog, but the epithelium is composed of the same stratified flat epithelium and multi-

lined ciliated epithelium as in adult dog, the distribution of the two kinds of epithelium being also much the same. The height of the epithelium is not much different from that of adult dog, being considerably tall. In the latter period of fetal life, the development of taste-buds is also considerable and their distribution is analogous to grown-up dog, being richest on the laryngeal side of epiglottis, decreasing in the order of plica ventricularis, the inner side of cartilago arytaenoides and rima glottidis having stratified flat epithelium.

The nerve elements entering the mucous membrane of larynx of canine fetus are behind that of adult dog, especially in the extreaepiglottid areas, but the distribution is little different. Plexus submucosus and plexus tunicae propriae are markedly well-developed on the laryngeal side of epiglottis (Fig. 1), very poor on the pharyngeal side thereof, poorer in plica ventricularis, pars intercartilaginea rimae glottidis and pars inferior laryngis in the order named, and poorest in pars intermembranacea rimae glottidis and ventriculus laryngis.

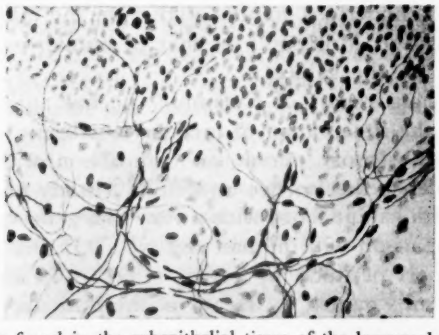

Fig. 1. Plexus found in the subepithelial tissue of the laryngeal side of epiglottis. Canine fetus in last stage. Seto's impregnation. X 400, reduced to ½.

Similar to that in grown-up dog, the development of sensory fibres and their terminations in larynx is parallel to the development of the subepithelial plexus. The laryngeal side of epiglottis is richest in sensory fibres, its pharyngeal side being very ill-provided. In plica ventricularis, pars intercartilaginea and pars inferior laryngis the development of sensory fibres is intermediary and very poor in pars intermembranacea and ventriculus laryngis.

Koizumi[4] has previously reported on the existence of sensory terminations Type II related with Hering's blood pressure falling reflex in the laryngeal mucous membrane of adult dog, but in canine fetus, we could detect no formation of Type II termination, not to sepak of Type I. It

is presumed that these terminations Type II are gradually formed after the birth.

Corpusclar terminations are rarely developed in the embryonic stage, even in man, as has been demonstrated by many a study in this laboratory. Much the less the existence of such terminations in canine fetus had been imaginable, since the nerve elements in the mucous membrane in canine larynx, even by grown-up dog, are so incomparably behind that of man in development. In fact, no complex sensory terminations, such as corpusclar terminations, have been observed anywhere in the mucous membrane of larynx, including the best-innervated laryngeal side of epiglottis. So, these complex terminations must have their initiation after the birth.

Thus the sensory terminations in the mucous membrane of larynx in canine fetus are represented by unbranched and brnached terminations of simple structure.

The sensory fibres are composed of medullated, fine or thick fibres, the latter frequently forming neuro-fibrillar dissolutions in their course. These medullated fibres, after losing their myelin, end sharply or sometimes bluntly, without branching in unbranched terminations, or in branched terminations, after branching into 2, 3 or sometimes more rami, which run various winding courses, as shown in Fig. 2. These terminations are generally simpler in construction than those in grown-up dog, but on the laryngeal side of epiglottis, sometimes the structure is more complex, either formed into arborized or into plexus-like terminations. Some of these terminal rami penetrate into the epithelium, to end in intraepithelial fibres, as described in the following.

Intraepithelial fibres by canine fetus are well-developed on the laryngeal side of epiglottis (Fig. 3), well-nigh coming up to those in adult dog, similar to the results obtained by Momono in his study on tenth month human embryo, but the development is somewhat lower than in grown-up dog in plica ventricularis and pars intercartilaginea and poorest in pars inferior laryngis, ventriculus laryngis and pars intermembranacea.

These intraepithelial fibres are divided into smooth-surfaced fine fibres with little change in size and thicker fibres showing conspicuous change in size, the latter being far fewer than in adult dog. The intraepithelial fibres also may be classified into branched and unbranched. Their course in the epithelium is very irregular, as in adult dog, and end sharply or sometimes bluntly, at various height in the epithelium (Fig. 4).

The sensory fibres supplying the taste-buds are represented by fine and thick fibres, as is the case by adult dog, their development in general being nearly equal to those in adult dog. However, the terminations are simpler in type, fewer typical glomerular or plexus-like terminations

Fig. 2

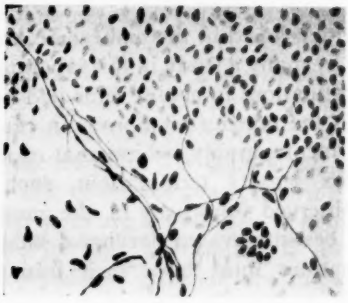

Fig. 3

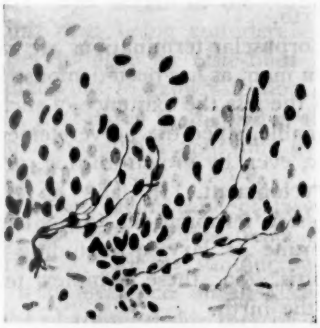

Fig. 2. Simple branched terminations formed beneath the epithelium of the laryngeal side of epiglottis. Same fetus. Same staining. X 400, reduced to ½.

Fig. 3. Intraepithelial fibres found in plica ventricularis. Same fetus. Same staining. X 500, reduced to 2/3.

Fig. 4

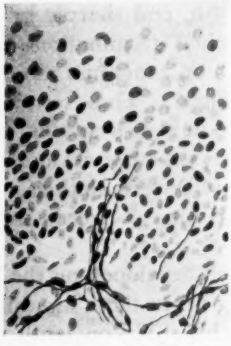

Fig. 5

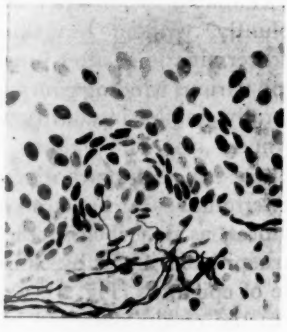

Fig. 4. Intraepithelial fibres found on the laryngeal side of epiglottis. Same fetus. Same staining. X 400, reduced to 2/3.

Fig. 5. Simple plexus-like termination originated in thick fibres formed closely under a taste-bud on the laryngeal side of epiglottis. An intragemmal fibre is seen running out. Same fetus. Same staining. X 500, reduced to 2/3.

being formed beneath the taste-buds, and intra- and extragemmal fibres are rarely formed. Fig. 5 shows the distribution of sensory fibres to a taste-bud on the laryngeal side of epiglottis. The thick sensory fibres penetrate closely to the base of the taste-bud to form a rather complex plexus-like ending, an intragemmal fibre being sent out therefrom. Such taste-buds well-provided with sensory fibres are found often enough, but in general, the sensory supply of the taste-buds is simpler even in the last days of the fetal life.

SUMMARY

The development of sensory nerve fibres in the mucous membrane of larynx of canine fetus in the last stage is weaker than that in grown-up dog, but the distribution is much the same, being richest on the laryngeal side of epiglottis and poorest in pars intermembranacea and ventriculus laryngis.

No formation of sensory terminations related with Hering's blood pressure falling reflex has been observed, neither of Type I nor Type II. These are presumed to be formed gradually after birth. The sensory terminations are in general represented by unbranched and simple branched terminations. Some of them frequently pass into intraepithelial fibres which are especially well-developed on the laryngeal side of epiglottis, so as to compare favorably with those in adult dog.

The formation of taste-buds in larynx is not much different from that in adult dog, no noteworthy difference existing between the developments of the sensory fibres to the taste-buds of fetal and adult dogs. However, their terminations are in fetus generally simpler than in adult in structure, intra- and extragemmal fibres being found only rarely.

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Structural Study of the Group Carbohydrate from Pig Stomach Mucus

9th Report*

By

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(Received for publication, September 1, 1953)

1) From a batch of pig stomach mucus was obtained a group carbohydrate (amino acid-polysaccharide complex) specimen, containing 14% L-fucose—Ch. I—in a manner already reported by Yosizawa.[1] By fractionation with alcohol was separated from it an alcohol-soluble part containing 10% fucose, which showed Group A activity at 6.4×10^5-fold dilution and Group O activity (measured with an eel serum) at 3.2×10^4-fold dilution—Ch. II. Further, this was heated with 1 *N* acetic acid at 100°C for 1 hour and for 3 hours, and Ch. III and Ch. IV were recovered respectively. Ch. III contained about 5% fucose but Ch. IV only a trace of this sugar. The Group A activity of Ch. II decreased to 1/16 by both the 1 hour and 3 hours heating with acetic acid of the substance, but the Group O activity to 1/8 and 1/32 respectively. Judging from the analyses (of the sugar components and iodine use), positiveness of the indirect Osaki-Turumi reaction and the periodate consumptions of Chs. I~IV, fucose in Ch. II partakes most probably in forming the non-reducing terminal disaccharide unit and the adjacent of the polysaccharide chain, replacing galactose of the other disaccharide units made up of acetylglucosamine and galactose. Moreover, the experiments with hot acetic acid suggested that fucose is not absolutely necessary for the Group A specificity.

2) Pig gastric mucus was divided into four fractions inclusive of the gastric mucin. All of the fractions were strongly Group A active and much less Group O active. They contained same kinds of sugar (glucosamine, chondrosamine, galactose, mannose and L-fucose) in the equivalent ratios within respective fractions, which resembled mutually between the fractions, so that the same group carbohydrate looked obtainable from any of the fractions. Next, group carbohydrate specimens containing chondrosamine, glucosamine, galactose and fucose were given by tryptic digestion as well as by scission with 1 *N* NaOH at 0°C of the mucin, but specimens similar to Ch. I in 1), which lacked chondrosamine and mannose, by applying 1 *N* NaOH to the mucin and another mucus fraction at about 20°C.

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* Details were read before the 25th General Meeting of the Japanese Biochemical Society, Tokyo, April 26–28, 1953.

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Histological Studies on Innervation of Lung of Human Embryo

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(Received for publication, January 19, 1953)

On account of the difficulty of nerve staining, the histology of the nerves in the lung was studied only by a few investigators, among them, Arimoto and Miyagawa,[1] Berkley,[2] Larsell,[3] Müller,[4] Ponzio[5] and Takino,[6] who, for the same reason, failed to obtain noteworthy results. However, in 1937, Hayashi[7] published a highly commendable and minute report on the innervation of lung of adult man and dog, which threw much licht on the obscure problem. On the other hand, even less study was expended on the genetics of the subject. In the last years of the preceding century, Retzius[8] reported study of 4th month human embryo under Golgi's method and Larsell and Dow[9] of 8th month human embryo stained with methylene blue, and that is about all we know, but these studies contain much questionable matter, inevitable in view of the imperfect staining methods then available.

In view of such a situation, I took my materials of research from 4th, 6th and 8th human embryos, and fixing them in 10% neutral formol for a long time, sliced them into 40 μ horizontal and sagittal sections, and staining them with Seto's silver impregnation, obtained a large series of very beautiful preparations. These preparations I subjected to a thoroughgoing microscopic research and arrived at the observations given hereunder.

Individual Views

To speak nothing of 6th and 8th month embryos, even in 4th month embryo, the nerve elements running through the lung hilus into the lung are remarkably well-developed, as are the other various parts of the body (Funahashi,[10] Iwasaki,[11] Yamada[12]), in particular, the formation of plexus being in a nearly accomplished state. It is difficult in adult to study the general innervation of lung in one section, but as in embryo, the lung is very small, much more so in 4th month embryo, the general

outline is easily caught in one sight.

I will preface my full report on the innervation with a few words on the histological genesis of lung, seeing that even in the noted text-books, little is found in detailed description on the subject. First of all, the bronchus and the major bronchial branches are conspicuously short but are complete in their histological conditions in general, though showing a very undifferenciated state of development, even in 4th month embryo. So, in the bronchus, epithelium is represented by the multi-lined ciliated epithelium, and tunica propria and tela submucosa are also observable. Only the development of glands is belated, mere gland ducts being seen, but no gland bodies. The surface of mucous membrane is provided with comparatively well developed longitudinally running folds, as much in the cartilaginous as in the uncartilaged parts. The development of cartilages around the mucous membrane is also remarkable, and the circular smooth muscle layer in pars membranacea is also well formed. As the later stage of embryonic life, as in the 6th and 8th month, is reached, the bronchus increases in length and their histological picture approximates maturity, while the mucous membrane in the cartilaginous parts more or less loses the folds and becomes comparatively smooth on the surface. The gland bodies appear in the 6th month and are nearly perfected by the 8th month.

The bronchial branches are in the 4th month embryo provided with numerous mucous folds much taller than in the bronchus on the surface, while the epithelium is consisted of well-developed 2 to 3 lined ciliated epithelium. In submucosa, circular smooth muscle fibres were conspicuously formed, but here also the glands were found ill-developed. These branches divide again into almost uncartilaged sub-branches after a short extension, which are covered with folded mucous membrane and 1 or 2 lined ciliated epithelium. These subranches branch out into bronchuli consisting of foldless single-lined columnar epithelium, which finally ramify into alveolar ducts of single-lined cubic epithelium. At this stage, no alveoli are formed as yet in the lung.

In 6th and 8th month embryos, these broncial branches gradually increase in size as well as length and ramify into numerous branches, which become smaller in diameter and poorer in folds, as the distal parts are reached. The glands and the smooth muscle fibres appear also simultaneously, so that in 8th month embryo, the histological picture of the lung as a whole almost gives a completed appearance. The uncartilaged small branches shortly branch out into bronchuli, to divide again into alveolar ducts, and end in alveolar sacks. After the 6th month, the alveolae begin to appear markedly, and in 8th month, they are seen growing up in every part.

The nerves to the lung are known to consist of sympathetic nerves and parasympathetic nerves from the vagus, and Hayashi[7] succeeded in confirming the mixture of comparatively large quantity of sensory nerve fibres in this vagus. The nerve elements form plexus pulmonalis ventralis consisting of considerably large bundles and still stronger plexus pulmonalis dorsalis, both of which run their course into the lung through the lung root along the circumference of the major bronchial branches. This course is especially clearly observable in 4th month embryo.

These plexuses have been proved in mammals by Glaser,[13] Stöhr,[14] Takino[6] and etc., who classified them into plexus extra- and intracartilagineus. Hayashi[7] also observed the plexus well developed in human and canine lungs, and named the former the ground plexus and the latter the inner secondary plexus. The plexus consisting of minor nerve bundles originating in the ground plexus and formed on the circumference thereof, he called the outer secondary plexus, and asserted that minute nerve fibres run out of this plexus into the lung substance with small blood vessels. Hayashi also describes the so-called subepithelial plexus originating in the inner secondary plexus and extending longitudinally in the subepithelial connective tissue. Certainly, Berkley,[2] Ponzio[5] and Glaser[13] did not miss to give their observations on this plexus, but their descriptions were all to sketchy.

In my specimens of human embryos, the plexus formation in the lung was very remarkable, especially to a surprising maturity in 4th month embryo already, as mentioned above. Sympathetic nerve bundles consisting of minute fibres and vagus nerve bundles consisting of parasympathetic fibres and containing stout sensory fibres run into the lung, along the bronchial branches, forming the ground plexus (Hayashi). In their course, the plexus sends out finer bundles to the interior of the cartilages to form the secondary plexus, whence extremely minute fibres extend into the subepithelial tissue, finally to pass into the minute mesh-like subepithelial plexus (Fig. 1). However, the plexus corresponding to Hayashi's outer secondary plexus was not formed in my specimens.

The above described plexus become gradually poorer in development, as the ends of the bronchial branches are approached and the respiratory tracts decrease in size. By the small bronchial branches without cartilage, only very weakly developed pleuxs surrounding them is found. However, nerve elements often are found running to the circumference of the bronchuli as well as the laveolar ducts (Fig. 2).

Finally, very minute plexus is often found also in the connective tissue of the basis of pleura visceralis. This plexus frequently contain 2 or 3 nerve cells and a few sensory nerve fibres, as in adult (Hayashi).

It has been accepted that there existed nerve cells in the lung, over

Fig. 1

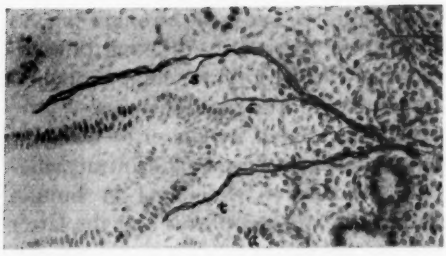

Fig. 2

Fig. 1. Plexus spreading out around a bronchial branch in 4th month human embryo, consisting of thin vegetative fibres and thick sensory fibres. t vegetative terminalreticulum; s unbranched sensory terminations; a alveolar duct. Seto's silver impregnation, X 300, reduced to 2/5.

Fig. 2. A bifurcated sensory termination accompanied by fine vegetative fibres found in lung parenchyma in 4th month human embryo. a alveolar duct; g. small ganglion along the plexus around a bronchial branch. Same staining, X 300, reduced to 2/5.

since Remak discovered them in 1844, but their microscopic structure had not been adequately clarified, till Hayashi threw light on the subject in his study on human and canine adult lungs. According to him, these nerve cells are found in group or ganglion formation mainly in the ground plexus, and also partly in the secondary plexus, classified into two kinds of cell groups with and without conspicuous connective tissue capsule. The ganglion formation becomes smaller in size as the bronchial branches become smaller. In rarer cases, the cells are found in twos or threes, or even in solitary existence. Such cases are mostly found in the interstitial connective tissue or visceral pleura.

Müller first proved that the nerve cells in the lung are multipolar, in 1910, and the fact has been confirmed by Larsell[3] and Glaser[13] since then. Hayashi[7] in his study classified them into the cells Type I and Type II of Dogiel, as all ympathetic nerve cells in general, and showed that the majority of the nerve cells in the lung belong to the Tpye II, while the cells Type I are very few, unlike the cells in the other organs. In the canine lung, he says, fenestrated nerve cells belonging to the cells Type II are found very often. In the human embryo, I found the following about the nerve cells in the lung. The distribution of the cells is much the same in embryo as in adult. The nerve cells are in the main

grouped in a number in or along the nerve bundles of the ground plexus, being represented as capsulated or noncapsulated ganglia, but sometimes also some cells are found scattered solitarily or in twos and threes. Not rarely, a very limited number of cells are found in the interstitial connective tissue and the subpleural plexus.

As cited above, Retzius[8] spoke some words on the innervation of the lung of a 4th month embryo, but perhaps due to the imperfect staining he used which was not effective enough to stain the cells, he makes no comment on the nerve cells. But Larsell and Dow[9] succeeded in discovering ganglia in the 8th month embryo and states that all nerve cells are multipolar. I have observed the following on the subject.

In 4th month embryo, the nerve elements were stained with perfect clarity, nerve cells as well as nerve fibres being observable very distinctly. As shown in Fig. 3, the nerve cells are very large in comparison with mantle cells, and are provided with very large round cell nucleus. The protoplasm occupies a rather small territory, staining red under my staining, and often containing extremely minute neurofibril network. The shape of the cells is generally round and infantile, but some cells are observed with 2 or 3 minute processes, differenciated into stellar shape. However, no such typical multipolar cells as are common by adult are observable. So the nerve cells in the lung of 4th month embryo are mostly represented by unprocessed very young cells, which however show their characteristics in that they have over-sized cell nucleus, their protoplasm stains red, and frequent formation of minute fibril network is found therein.

In 6th month embryo, the nerve cells in the lung show a perceptible increase in their size, the formation of the fibril network in the protoplasm becomes more marked, and the number of the processes rises above that in 4th embryo. In 8th month embryo, such growths become even more remarkable, the processes become more developed and the formation of neurofibril net is more accentuated. However, the number of processes is still far smaller than in adult man and dog (Hayashi[7]), so that the distinction between Types I and II of Dogiel is as yet impossible (Fig. 4). Of course no fenestrated cells are anywhere observable.

As stated in the above, the nerve cells in the lung are showing their peculiarity early in the 4th month, though rather infantile in shape, the specialized development progresses as the 6th and the 8th month are passed and the formation approaches perfection as multipolar cells, though very simple in nature. In short, the nerve cells in the lung in the embryonic stage are in the course of development, and the completed formation is to come on after birth.

The termination of vegetative nerve fibres, as has been acknowledged by many investigators recently, is represented by Stöhr's terminalreticu-

Fig. 3

Fig. 4

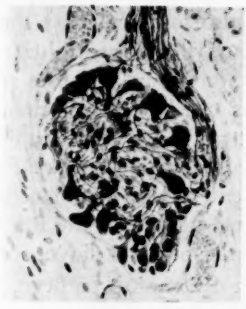

Fig. 3. A small ganglion accompanying a minute nerve bundle around a bronchial branch in 4th month human embryo. Nerve cells are of very young type. Same staining, X 500, reduced to ½.

Fig. 4. A round ganglion along a nerve bundle running around a bronchial branch in 8th month human embryo. Refer to the text for particulars. Same staining, X 300, reduced to ½.

lum, and this fact has been confirmed by many studies in this laboratory. Hayashi[7] reports that the termination of vegetative fibres in the lung, both in adult man and dog, is represented by terminalreticulum, in bronchial glands, in bronchial muscles, in tunica propria, in blood vessel walls as well as in ganglia.

In my study on the embryonic lung, I also found that the termination of vegetative fibres was represented by Stöhr's terminalreticulum, I could not find any free sharp or knob-like endings allegedly found by former neurologists in the bronchial muscles, nor the free branched endings in the glands and smooth muscles in the lung of 8th month human embryo seen by Larsell and Dow[9].

The vegetative terminalreticulum in the lung of human embryo, as in the other organs (Yamada[12] and Yabuki[13]), is formed in the very early age of 4 months. As seen in Figs. 1 and 5, the vegetative fibres pass into the cord-like arrangement of the network of further indivisible extremely minute fibrils i.e. Stöhr's terminalreticulum. This always comes into mutual anastomosis and never ends free. Besides, it contains Schwann's cell nuclei in places, and against the supplied cells, for example gland cells, smooth muscle cells etc., it stands in close contact control from every side. Often it penetrates right through connective tissue cells.

It was supposed from early times that in the lung sensory fibres may be distributed also. Larsell[3] reported discovery of such nerve fibres in mammal lungs, Larsell and Dow[9] in the lung of 8th human embryo and Glaser[13] in the mucous membrane of human bronchial brnaches. But

as had been doubted by Stöhr[14] and Hayashi,[7] the so-called sensory terminations, as found by the above investigators, seem to have been artefacts generated by the imperfect staining methods used by them. However, the sensory termination Type I connected with blood pressure falling reflex found by Sunder-Plassmann[16] in the muscular layer of the bronchial branches and the sensory terminations discovered by Hayashi[7] in various parts of the lung are deserving full credence, on seeing their illustrations.

In succession to the discovery of Hayashi[7], I also succeeded in finding sensory terminations in the lung of human embryo in many parts. This has further confirmed their existence in human lung. These sensory terminations in the fetal life, however, show in most cases very unspecialized simple formation, as is the case with other organs (Funahashi[10], Iwasaki[11], Yamada[12]), no such complex branched terminations with knobs at each terminal branch, as described by Larsell and Dow[9] being found anywhere. I will outline my observations in the following.

The sensory fibres in adult human lung consisting of conspicuous myelin and stout axon, their histological distinction from the vegetative fibres consisting of non-medullated fibres is clear enough. The distinction is easy in the embryo already, but the formation of myelin being as yet indistinct especially in 4th month embryo, the nerve fibres must be distinguished by the thickness of the axons. But as the termination of vegetative fibres is represented by the terminalreticulum and that of sensory fibres by free terminations, the distinction at their terminal parts is not at all difficult. The thick sensory fibres belong to the vagus nerve, as mentioned above, and are smaller in number than the non-medullated fibres. They first pass over from the ground plexus into the secondary plexus, then separate from the nerve bundles, finally to end in free endings (Fig. 5).

In adult, the sensory endings are located in the bronchial muscle layer, in the subepithelial connective tissue, and in rarer case in the interstitial connective tissue, as complex branched terminations, and in the epithelium as intraepithelial fibres (Hayashi[7]). The peculiarly shaped Type I endings connected with the blood pressure falling reflex (Sunder-Plassmann[16]) have also been observed, but the sensory endings in the embryonic lung are incomparably smaller in scale than those in adult. They are infantile and are in the course of development, especially in 4th month embryo. In 6th and 8th month embryos, the differenciation is still very poor, the endings being represented by infantile types.

As the sensory endings in the embryonic lung are extremely simple in scale, as described in the above, specialized endings, such as of Sunder-Plassmann, are not yet formed at all. They seem to be gradually formed after birth.

The sensory fibres in the embryonic lung all end branched out into 2 or 3 branches or unbranched sharply, and mainly in the muscle layer or subepithelial tissue of the bronchial branches (Fig. 1). Such endings are also often found in the connective tissue around the bronchuli (Figs. 2 and 5).

The intraepithelial fibres in the embryonic lung are also very poorly developed, no such complex branched endings with knobs, as allegedly found by Larsell and Dow[9] in 8th month embryo, being ever observed. In fact, intraepithelial fibres are found as unbranched fibres in the multilined ciliated epithelium of the thick bronchial branches, and also often enough in the epithelium of the thin branches (Fig. 6). Their quantity is also far lower than in adult.

Fig. 5

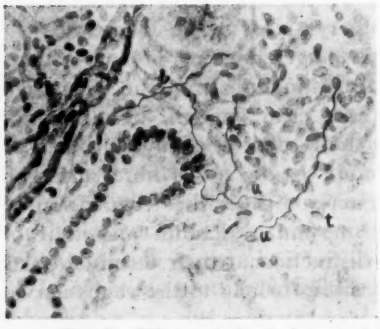

Fig. 6

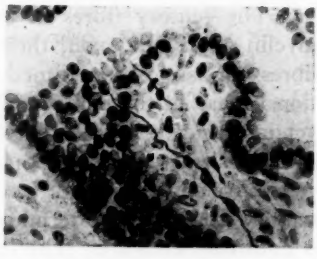

Fig. 5. Bifurcated simple branched (b) and unbranched sensory terminations (u) and vegetative terminalreticulum (t) found around a bronchulus in 4th month human embryo. Same staining, X 600, reduced to ½.

Fig. 6. Unbranched intraepithelial fibres penetrating into the epithelium of a bronchial branch in 6th month human embryo. Same staining, X 800, reduced to ½.

Recently, Yabuki[15] of this laboratory made a minute histological study on the distribution of the aorta nerve in human embryo and succeeded in obtaining many new observations. According to him, these sensory fibres connected with the blood pressure falling reflex are distributed far wider in embryo than in adult, extending in part as far as the root of a. pulmonalis. Their endings consist of terminal branches with very peculiar change in size, and reach in 4th month embryo into the media, and some even into the intima, but fall into degeneration in the later stage, so that in 8th month embryo, the endings in the media almost disappear and only some endings are left in the externa. A few investigators have indeed mentioned sensory endings in the walls of a. pulmonalis, but none had given the description thereon in detail with

good illustrations.

In consideration of the above, I was led to find in human embryo not only in the root of a. pulmonalis, but also in its distal parts running intrapulmonally such sensory endings as found by Yabuki,[15] conducted a thorough research for it, and arrived at the following observations. As a. pulmonalis penetrates into the lung and loses in size by ramification, the number of the sensory fibres running along its externa also diminishes apace. In 4th month embryo, the endings penetrating the externa into the media are conspicuously present (Fig. 7), as found by Yabuki, but in 6th and 8th month embryos, few of them are found in the media, and only a limited number even in the externa.

The sensory endings found in the media of a. pulmonalis of 4th month embryo are much simpler in construction in comparison with those described by Yabuki in the root of the artery, being represented as simple branched endings dividing only into 2 or 3 branches, but as the specific construction is much the same, it is not difficult to believe their origin in the same aorta nerve. The terminal fibres of these endings are namely rich in peculiar change in size, accompanying specific nuclei, run circularly along the muscle fibres of the media and end always bluntly, as shown in Fig. 8.

Fig. 7

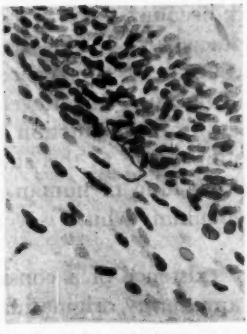

Fig. 8

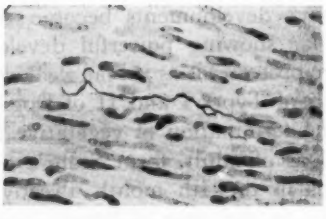

Fig. 7. A branched sensory termination penetrating the media of pulmonary artery in lung in 4th month human embryo. Same staining, X 700, reduced to $\frac{1}{3}$.

Fig. 8. A sensory termination with peculiar form found in the media of pulmonary artery in lung. 4th month human embryo. Same staining, X 800, reduced to $\frac{1}{3}$.

Larsell and Dow[9] described sensory endings of very complex structure in the externa of a. pulmonalis in the lung of 8th month embryo, but such complex endings have never been found in my specimens. I suppose they were only artefacts created by the methylene blue staining. A few

investigators describe sensory endings existing also in the walls of v. pulmonalis, but in the pulmonal vein of the embryos I studied, I found few sensory fibres and no formation of sensory endings was confirmed.

Summary

The ground plexus around the bronchus and bronchial branches, the secondary plexus in the inner side of the bronchial cartilages and the plexus under the bronchial epithelium are in remarkable formation in 4th month embryo. These plexuses become poorer in development as the bronchial branches approach their ends, and in the small uncartilaged bronchial branches, there are found only weakly developed plexus around their walls. Some nerve elements are observed penetrating into the circumference of the bronchuli and alveolar ducts.

The distribution of the nerve cells in the lung of human embryo is much the same as in adult. They aggregate along or in the nerve bundles of ground plexus, to form capsulated or non-capsulated ganglia, but sometimes sporadic existence of 1 or 3 cells is also observable.

The nerve cells in the lung of 4th month embryo are large cells containing ober-sized cell nucleus and protoplasm staining red under my silver staining and including extremely minute fibril net. In general, the form is as yet round and infantile, but some have already grown a few processes and appear stellar in shape. By the 6th month, these cells show some increase in size, the fibril net-work becomes more marked and the number of cells with processes also rises somewhat. In the 8th month, the above developments become more accentuated, the processes in particular showing powerful development. However, the development of nerve processes is as yet far weaker than in adult, the distinction between the cells of Types I and II of Dogiel being impossible at this stage.

The termination of vegetative fibres, in the lung of human embryo as well as of adult, form Stöhr's terminalreticulum, which is very conspicuous in the 4th month already.

Also in the lung of human embryo, the existence of a considerable number of sensory fibres and their endings, apparently originating in the vagus nerve, is observable. However, the endings are as yet of simple nature, no such complex endings as found in adult lung being represented. Consequently, such complex sensory endings as those connected with blood pressure falling reflex (Sunder-Plassmann) have never been found.

Such infantility in terminal formation is of course the most marked in 4th month embryo. The sensory endings are represented by unbranched or simple branched terminations in the muscle layer or the subepithelial tissue of the bronchial branches. Intraepithelial fibres are found, but they also show unbranched or very simple branched formation

and are very small in number, as compared with those in adult lung.

The distribution of the aorta nerve of sensory nature concerned with blood pressure falling reflex is much wider in the embryo than in the adult, extending partially to the root of a. pulmonalis (Yabuki[15]), and as I have found, to its distal prolongation. The sensory endings seen here are much simpler in scale than those in the root of a. pulmonalis but the same in general structure as the latter, being represented by simple branched endings conssisting of 2 or 3 branches showing peculiar change in size running along the muscle fibres in the media. These are found in good development in 4th month embryo, but fall into degeneration in the later months.

No existence of sensory terminations may be found around the pulmonary vein.

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A Hypothesis on the Spacial Configuration of Group A and Group B Substances*

By

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The study of Yosizawa[1] indicates that the molecule of the Group A carbohydrate from pig mucus forms a coil of rings made up each of six hexose units, in so far as the first seven unit disaccharides (an acetylglucosamine-β-galactoside) beginning with the reducing are concerned. Even by splitting off of fucose molecules, its group specificity was not altered (Cf. Masamune and Yosizawa[2]). For this and other reasons, it is plausibly assumed that the last two disaccharide units are constructed by replacing β-galactose of the disaccharide units above with β-L-fucose so as to complete the final (third) ring possessing a bare uneven surface which is partially similar to the corresponding surface of the second ring. The coiling of the carbohydrate chain must be left-handed, because, thus only, the peptide chain bound α-N-glycosidically at one end can make a close lateral attachment to the first ring, bestowing a regular and rigid internal structure to the coil by virtue of many hyrogen bonds. The other cut end of the coil are then well fitted to α-agglutinin with which it combines specifically. If the material were a Group B substance, the coiling would be right-handed in order that the first ring may be shielded by the peptide thread. In the Group B lipoid of pig lung, a much less potent substance, the carbohydrate moiety contains only six hexose units (Cf. Masamune *et al.*[3]) and can form one plane ring at most, if the mode of union between two adjacent hexose units is same as that in the above group substance. Of the two broad surfaces of the ring, one is open to bind β-agglutinin. Since the peptide chain is not sufficiently long and the essential lipid moiety fills up the shortage, the other surface of the ring is not fastend well, entailing weakness of group activity, and the conversion of the lipid into a Group A substance (α,β-rearrangement of the N-glycoside) readily takes place. Probably most natural Group A and Group B substances have a spacial configuration related to above, although any colloidal substance carrying a surface, suited to α- or β-agglutinin and rigid, might be specifically group active.

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* Details were read before the 25th General Meeting of the Japanese Biochemical Society, Tokyo, April 26-28, 1953.

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A Survey on *Bacillus Chitinovorus* Benecke in Japan

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Benecke[1] was the first to report the isolation of Bacillus chitinovorus, a bacillus which decomposes chitin, from the sea water of Kiel Bay in 1905. Later, the bacillus was collected and studied mainly in Germany and in the United States, taking as sources various materials such as sea mud, sea water, fresh lake water, soil, compost, crustacea etc. However, the study of this bacillus has never been started in Japan, so I undertook the isolation of the bacillus from materials such as sea water, lake water and toads for investigation of its bacteriological nature.

Experimental

I) Collection of Material

The material used includes mud or sand, put into sterilized flasks, which was collected from the sea or lake beds of about 30 cm. in depth in the Tohoku, Kanto and Chubu districts and also mucus-like substance obtained from the surface of the stomach of toads collected in Tokyo. The material was collected during the summer season from May to September in 1950 and 1951.

II) Isolation of the Bacillus

The material above described was mixed with so-called Benton's[2] phosphate buffer medium with chitin added and incubated at 25°C. If chitin was softened and digested during a period of three weeks, the turbid fluid of the medium was cultivated on bouillon agar plate at 25°C. for 24 hours. To obtain pure strains of the bacillus the colonies were fished and cultivated on bouillon agar slants subsequently. Before the experiment, the chitin digesting nature of the isolated strains was confirmed by using Benton's medium. The localities of collection, number of toads collected, number of strains are shown in Table I, i.e. 164 strains were collected from 11 sea beds in the Kanto and Tohoku districts, 37 strains from 9 lake beds in the Kanto and Chubu districts and 10 strains

TABLE I

Collection No.	Strain No.	Place of Collection		
1	1～ 7	Sugita beach (Yokohama)	Tokyo Bay	Salt water
2	8～ 12	Kanasawahakkei beach		
3	13～ 32	Kemigawa beach		
4	33～ 49	Inage beach		
5	50～ 62	Yuigahama beach	Sagami Bay	
6	63～ 72	〃 〃		
7	73～ 84	Itô beach (Izu)		
8	85～104	Shikinejima beach	Pacific Ocean	
9	105～114	Ishinomaki beach	Ishinomaki Bay	
10	115～124	Mangokuura beach		
11	125～134	Onagawa beach	Onagawa Bay	
12	135～144	Matsushima beach	Matsushima Bay	
13	145～154	〃 〃		
14	155～164	Aomori beach	Aomori Bay	
15	165～174	Lake Kawaguchi	Yamanashi Prefecture	Fresh water
16	175～179	Lake Shôjin		
17	180～184	Lake Chûzenji	Tochigi Prefecture	
18	185～187	Lake Taishô	Nagano Prefecture	
19	188～189	Lake Tashiro (Kamikôchi)		
20	190～191	Lake Myôjin		
21	192～196	Lake Kasumigaura	Ibaragi Prefecture	
22	197～199	Reservoir Murayama	Tokyo	
23	200～201	Reservoir Yamaguchi	Saitama Prefecture	
24	202～211	Stomach of the Bufo vulgaris (5) in Tokyo		Toads

(5)=Animals number of toads

from the mucous membrane of the stomach of 5 toads from Tokyo.

III) General Biological Nature of the Bacilli Collected

A) Method of experiment. The general biological nature of 211 strains isolated in the manner mentioned above was examined by the following 16 tests: the shape and size, Gram stain, flagella, the decomposition of milk, the liquefaction of gelatine, the color shown on potatoes, the fermentation of carbohydrates such as glucose, maltose, mannit, sucrose, lactose, starch and cellulose, the nitrate reduction, the formation of indol and hydrogen sulphide. Most of the examinations were carried out after Benton.[2] The staining of flagella followed Imai and Hidaka's method. As for the indicator for the media of various kinds of sugar and milk, bromthymolblue was selected. The formation of gas was examined by means of Durham's tube and as for the other tests not described here the routine method was used.

B) Results of experiment. 1) Sea water strains. After examination of 164 strains as described previously, these were classified into 40 types as shown in Table II. The type number was determined chiefly according to the behavior of the strain in the fermentation of carbohydrates and secondarily by means of the other tests. The strain numbers of each type are given in Table III. They may be summarized as follows: All

of them were Gram negative. As for their shape and size they were divided into 3 groups. a) 15 types belonged to the group of small rods ($0.3\mu \times 1.0\mu - 2.0\mu$), b) 18 types were included in the group of rods ($0.8\mu \times 2.0\mu - 3.0\mu$), and c) the other 7 types in the group of plump rods ($1.0\mu \times 2.0\mu - 7.0\mu$). All of them possessed peritrichous flagella. As regards the decomposition of milk, 15 types gave no reaction, 18 types peptonized, 2 types produced alkaline and 5 types produced alkaline and peptonized. 16 types liquefied gelatine. Indol producing strains were limited to Type I only. All gave a negative reaction in the cellulose dissolution test and in 20 types the nitrate reduction test was positive. The colors shown on potatoes were of five kinds, that is, 20 types were brownish, 7 types were orange, 8 types were yellow, 2 types were putty color and 3 types were abundant pink. As regards the fermentation of sugar, Type I decomposed any kind of sugar other than starch, Type II decomposed glucose, maltose and mannit with simultaneous gas formation, which was also seen in type I and not in the others. Types III-V produced acid from lactose, sucrose, maltose, mannit, glucose and starch; Types VI-X decomposed 5 kinds except lactose; Types XI and XII decomposed 4 kinds except mannit and lactose, and Types XIII and XIV decomposed 4 kinds except lactose and starch respectively. Types XV and XVI decomposed glucose, maltose and sucrose; Types XVII and XVIII glucose, maltose and starch; Types XIX and XX glucose, sucrose and starch; Types XXI and XXII glucose and maltose, Type XXIII glucose and sucrose. Types XXIV and XXV only glucose and Types XXVI and XXVII only sucrose. Types XXVIII-XL did not decompose any of the six kinds of sugar. No strains formed H_2S.

2) Fresh water strains. As in the case of (1) 37 strains were classified into 16 types as shown in Table IV and the strain numbers corresponding to each type are given in Table V. All of them were proved as Gram negative and their shapes and sizes included 1 type of small rods ($0.3\mu \times 1.0\mu - 2.0\mu$) and 15 types of rods ($0.8\mu \times 2.0\mu - 3.0\mu$). All of them had peritrichous flagella, as regards the decomposition of milk, 2 types showed no change, 11 types peptonized and 3 types produced alkaline. There were 5 types which liquefied gelatine and 4 types which produced indol. The coloration on potatoes were the following three kinds, brownish with 7 types, orange with 2 types and putty color with 7 types. None of the strains decomposed cellulose and 5 types of them produced nitrites from nitrate.

Subsequently, classifying strains on the basis of sugar fermentation as in case of (1), those belonging to Type I decomposed all kinds of sugar with the gas formation except the case of starch where gas formation was absent, and Types II and III decomposed any kind of sugar other than

TABLE II

Type	Gram	Shape	Flagella	Milk	Gelatin	Potato	Glucose	Maltose	Mannit	Sucrose	Lactose	Starch	Cellulose	Indol	Nitrate-reduction	H_2S
I	–	rods 0.8×2.0–3.0μ	peritric	peptonized	–	brownish	AG	AG	AG	AG	AG	–	–	+	+	–
II	–	plump rods 1.0×2.0–7.0	"	no change	–	orange	AG	AG	AG	–	–	–	–	–	–	–
III	–	rods 0.8×2.0–3.0	"	peptonized	–	brownish	A	A	A	A	A	A	–	–	+	–
IV	–	small rods 0.3×1.0–2.0	"	"	+	"	A	A	A	A	A	A	–	–	–	–
V	–	rods 0.8×2.0–3.0	"	"	+	orange	A	A	A	A	A	A	–	–	–	–
VI	–	"	"	no change	+	putty color	A	A	A	A	–	A	–	–	+	–
VII	–	plump rods 1.0×2.0–7.0	"	alkaline	+	yellow	A	A	A	A	–	A	–	–	+	–
VIII	–	small rods 0.3×1.0–2.0	"	no change	+	"	A	A	A	A	–	A	–	–	+	–
IX	–	rods 0.8×2.0–3.0	"	peptonized	–	brownish	A	A	A	A	–	A	–	–	–	–
X	–	small rods 0.3×1.0–2.0	"	no change	+	orange	A	A	A	A	–	A	–	–	–	–
XI	–	"	"	"	+	"	A	A	–	A	–	A	–	–	+	–
XII	–	"	"	peptonized	+	brownish	A	A	–	A	–	A	–	–	–	–
XIII	–	"	"	no change	–	"	A	A	A	A	–	–	–	–	–	–
XIV	–	rods 0.8×2.0–3.0	"	peptonized	–	"	A	A	A	A	–	–	–	–	+	–
XV	–	small rods 0.3×1.0–2.0	"	"	+	yellow	A	A	–	A	–	–	–	–	–	–
XVI	–	rods 0.8×2.0–3.0	"	"	–	"	A	A	–	A	–	–	–	–	+	–
XVII	–	plump rods 1.0×2.0–7.0	"	no change	–	"	A	A	–	–	–	A	–	–	+	–
XVIII	–	small rods 0.3×1.0–2.0	"	peptonized	–	putty color	A	A	–	–	–	A	–	–	–	–
XIX	–	rods 0.8×2.0–3.0	"	no change	+	yellow	A	–	–	A	–	A	–	–	–	–
XX	–	small rods 0.3×1.0–2.0	"	"	–	"	A	–	–	A	–	A	–	–	+	–
XXI		rods	"	peptonized	+	brownish	A	A	–	–	–	–	–	–	–	–

XX	–	small rods 0.3×1.0–2.0	,,	,,	–	,,	A	–	–	A	–	A	–	–	+	–
XXI	–	rods 0.8×2.0–3.0	,,	peptonized	+	brownish	A	A	–	–	–	–	–	–	–	–
XXII	–	plump rods 1.0×2.0–7.0	,,	no change	+	,,	A	A	–	–	–	–	–	–	+	–
XXIII	–	small rods 0.3×1.0–2.0	,,	alkaline peptonized	–	,,	A	–	–	A	–	–	–	–	–	–
XXIV	–	rods 0.8×2.0–3.0	,,	,,	+	,,	A	–	–	–	–	–	–	–	–	–
XXV	–	,,	,,	,,	–	,,	A	–	–	–	–	–	–	–	+	–
XXVI	–	small rods 0.3×1.0–2.0	,,	,,	–	orange	–	–	–	A	–	–	–	–	+	–
XXVII	–	rods 0.8×2.0–3.0	,,	no change	–	brownish	–	–	–	A	–	–	–	–	+	–
XXVIII	–	small rods 0.3×1.0–2.0	,,	peptonized	–	,,	–	–	–	–	–	–	–	–	–	–
XXIX	–	rods 0.8×2.0–3.0	,,	alkaline peptonized	–	,,	–	–	–	–	–	–	–	–	–	–
XXX	–	,,	,,	no change	–	,,	–	–	–	–	–	–	–	–	–	–
XXXI	–	plump rods 1.0×2.0–7.0	,,	peptonized	–	,,	–	–	–	–	–	–	–	–	–	–
XXXII	–	small rods 0.3×1.0–2.0	,,	no change	+	orange	–	–	–	–	–	–	–	–	–	–
XXXIII	–	rods 0.8×2.0–3.0	,,	peptonized	+	,,	–	–	–	–	–	–	–	–	–	–
XXXIV	–	,,	,,	,,	+	abundant pink	–	–	–	–	–	–	–	–	–	–
XXXV	–	plump rods 1.0×2.0–7.0	,,	no change	–	brownish	–	–	–	–	–	–	–	–	+	–
XXXVI	–	small rods 0.3×1.0–2.0	,,	,,	–	,,	–	–	–	–	–	–	–	–	+	–
XXXVII	–	rods 0.8×e.0–3.0	,,	peptonized	–	,,	–	–	–	–	–	–	–	–	+	–
XXXVIII	–	small rods 0.3×1.0–2.0	,,	,,	–	abundant pink	–	–	–	–	–	–	–	–	+	–
XXXIX	–	plump rods 1.0×2.0–7.0	,,	,,	–	,,	–	–	–	–	–	–	–	–	+	–
XL	–	rods 0.8×2.0–3.0	,,	alkaline	–	yellow	–	–	–	–	–	–	–	–	+	–

A=acid AG=acid and gas

TABLE III

Type	Strain No.
I	76.
II	140.
III	42.
IV	11. 47, 99, 134.
V	48, 130.
VI	1, 13, 52.
VII	9, 21.
VIII	155, 164.
IX	14, 106, 142.
X	20, 105, 159.
XI	12, 19, 161.
XII	115, 125.
XIII	64.
XIV	84, 162.
XV	136, 148, 149.
XVI	92, 113.
XVII	2.
XVIII	147.
XIX	107, 108, 110, 129.
XX	43, 49, 65, 116.
XXI	101, 112, 121, 135, 143.
XXII	3, 144.
XXIII	131, 137.
XXIV	53, 62.
XXV	94, 95, 98, 103.
XXVI	75, 123, 152.
XXVII	117, 128, 145.
XXVIII	5, 6, 8, 26, 31, 34, 37, 38, 50, 55, 63, 66, 80, 138, 163.
XXIX	24, 33, 54, 61, 81, 82, 93, 102, 104, 114, 120, 151, 156, 157, 160.
XXX	7, 25, 29, 44, 89, 111, 126, 158.
XXXI	4, 17, 57, 59, 122, 146.
XXXII	16, 22, 109, 119.
XXXIII	85, 100, 127, 153.
XXXIV	132, 133.
XXXV	18, 27, 28, 46, 87. 118, 124. 141.
XXXVI	30, 32, 45, 56, 67, 74, 78, 83, 86.
XXXVII	10, 15, 23, 35, 39, 41, 51, 58, 77, 96, 97, 154.
XXXVIII	36.
XXXIV	40.
XL	60, 68, 69, 70, 71, 72, 73, 79, 88, 90, 91, 139, 150.

starch, with accompanying gas formation, Types IV and V decomposed all kinds of sugar and Types VI and VII any kind of sugar other than lactose and starch with accompanying gas formation, respectively. Types VIII and IX decomposed glucose and sucrose, Type X only glucose and Types XI-XVI decomposed none of the 6 kinds of sugar. No strains produced H_2S as in case of sea water strain.

3) Strains isolated from bufo vulgaris. As in the case of (1) and (2), 10 strains were classified into six types as shown in Table VI and the strains belonging to each type are given in Table VII. All strains were proved as Gram negative and their shapes and sizes were divided

TABLE IV

Type	Gram	Shape	Flagella	Milk	Gelatin	Potato	Glucose	Maltose	Mannit	Sucrose	Lactose	Starch	Cellulose	Indol	Nitrate reduction	H_2S
I	–	rods $0.8\times2.0-3.0\,\mu$	peritritic	Peptonized	–	Putty color	AG	AG	AG	AG	AG	A	–	–	–	–
II	–	"	"	"	–	"	AG	AG	AG	AG	AG	–	–	–	–	–
III	–	"	"	"	+	"	AG	AG	AG	AG	AG	–	–	–	+	–
IV	–	"	"	"	+	brownish	AG	AG	AG	AG	A	A	–	+	+	–
V	–	"	"	"	+	orange	AG	AG	AG	AG	A	A	–	+	–	–
VI	–	"	"	"	+	brownish	AG	AG	AG	AG	–	–	–	+	+	–
VII	–	"	"	"	+	"	AG	AG	AG	AG	–	–	–	+	–	–
VIII	–	"	"	"	–	putty color	A	–	–	A	–	–	–	–	+	–
IX	–	"	"	"	–	"	A	–	–	A	–	–	–	–	–	–
X	–	"	"	"	–	"	A	–	–	–	–	–	–	–	–	–
XI	–	small rods $0.3\times1.0-2.0$	"	alkaline	–	"	–	–	–	–	–	–	–	–	–	–
XII	–	rods $0.8\times2.0-3.0$	"	"	–	orange	–	–	–	–	–	–	–	–	–	–
XIII	–	"	"	"	–	brownish	–	–	–	–	–	–	–	–	–	–
XIV	–	"	"	no change	–	"	–	–	–	–	–	–	–	–	+	–
XV	–	"	"	"	–	"	–	–	–	–	–	–	–	–	–	–
XVI	–	"	"	peptonized	–	"	–	–	–	–	–	–	–	–	–	–

A=Acid AG=Acid and Gas

TABLE V

Type	Strain No.
I	165.
II	168, 169, 172, 177, 183.
III	176.
IV	181.
V	180.
VI	198.
VII	199.
VIII	167.
IX	171, 184.
X	166, 170, 174, 182.
XI	173.
XII	178, 179.
XIII	188, 189, 196.
XIV	175, 185, 187, 192, 194, 195, 197, 200.
XV	193, 201.
XVI	186, 190, 191.

into two categories; that is, 5 types were rods ($0.8\mu \times 2.0\mu - 3.0\mu$) and 1 type was plump rods ($1.0\mu \times 2.0\mu - 7.0\mu$). All of them had peritrichous flagella. As regards the decomposition of milk, 5 types peptonized and 1 type was alkaline. The strains which liquefied gelatine and produced indol were classified into 5 types respectively. All strains showed negative results for the decomposition of cellulose and 2 types showed positive nitrate reduction. The colors shown on potatoes were observed as two kinds; that is, brownish with 1 type and orange with 5 types. As for the fermentation of sugars, Type VI produced acid from glucose and maltose, while the remaining 5 types fermented all of the six kinds of sugar submitted for examination.

Type I formed gas by decomposing any kind of sugar other than starch, and Types II, III and IV behaved in the same way by decomposing any kind of sugar other than lactose and starch; on the contrary gas formation was not seen in Types V and VI. In this case, there were no strains which did not ferment any of the six kinds of sugar. No strains produced H_2S.

4) Summary. On summarizing (1), (2) and (3), Type XXX of sea water strains was identical with Type XV of fresh water strains, and Types IV and V of fresh water strains were identical with Types II and III of the toads respectively. On summarizing the result the total number of types was given 59.

Summary and Discussion

Citing the bacteriological natures of bacillus chitinovorus obtained, Benecke[1] made a report on this bacillus isolated in Kiel Bay as follows:

TABLE VI

Type	Gram	Shape	Flagella	Milk	Gelatin	Potato	Glucose	Maltose	Mannit	Sucrose	Lactose	Starch	Cellulose	Indol	Nitrate-reduction	H_2S
I	−	rods 0.8×2.0−3.0 μ	peritritic	peptonized	+	orange	AG	AG	AG	AG	AG	A	−	+	−	−
II	−	"	"	"	+	brownish	AG	AG	AG	AG	A	A	−	+	+	+
III	−	"	"	"	+	orange	AG	AG	AG	AG	A	A	−	+	−	−
IV	−	plump rods 1.0×2.0−7.0	"	"	+	"	AG	AG	AG	AG	A	A	−	+	−	−
V	−	rods 0.8×2.0−3.0	"	"	+	"	A	A	A	A	A	A	−	+	+	+
VI	−	"	"	alkaline	−	"	A	A	−	−	−	−	−	+	−	−

A=Acid AG=acid and gas

TABLE VII

Type	Strain No.
I	202.
II	207.
III	204, 205, 206.
IV	209, 210, 211.
V	208.
VI	203.

Aerobic, Gram negative, no spore, flagella pritrichous and frequently showing the forms of mucous balls and chains; the bacillus produces acid from glucose and sucrose and makes use of lactose as a source of energy without the development of acid; liquefaction of gelatine and reduction of nitrate are seen, but there is no decomposition of cellulose and starch; and during culture he found no reduced substances such as glucosamine. Subsequently, in 1921 Folpmers[3] isolated two kinds of bacillus chitinovorus from the sea water of Kiel Bay, one of which had the same characteristic as Benecke's and the other had nothing different except that it did not liquefy gelatine.

In 1931 Steiner[4] reported that the mixed culture of bacilli collected from the water and soil of Alpine lake dissolved chitin in both states of aerobe and anaerobe culture. In the same year Rammelberg[5] reported that the bacilli isolated from compost had similar biological natures with those of bacillus chitinovorus and during the course of its growth the morphological character exhibited various changes as Benecke ob-

served previously. In 1935 Benton[2] isolated more than 250 strains of the chitin dissolving bacillus from many sources, such as lake water, mud and sand of lake beds, plankton, intestine of frogs, bats, trouts and ducks, leaf mould, and of stream beds, soil in pools, composts, rotten crawfish, chrysalises of wrigglers and bryozoa and classified them into 17 types according to their biological natures. Most of the strains showed Gram negative and polar flagella. He believed that they might not belong to the same genus and has emphasized that they were not described in Bergey's Mannual of Bacteriology edited in 1934. Then, Dravis and Skinner,[6] Zobell and Anderson,[7] Rittenberg, Anderson and Zobell[8] reported the isolation of a bacillus which dissolved chitin, but they did not make so detailed a description as Benton. In the same year, Hess[9] also isolated several kinds of the bacillus from the affected shell of a lobster which dissolved chitin. He reported that after a latent period of about two months, these bacilli dissolved chitin completely at 30°C. within two or three weeks and the dissolution always began with chitin on the surface of fluid. They did not decompose cellulose, liquefy gelatine and some of them produced gas from starch. Some strains which had the strongest chitinoclastic power bore a close resemblance to Benton's type I and others were identifical to type XIV. In 1938 Zobell and Rittenberg[10] isolated the chitin destroying bacillus from the water of the Antarctic Ocean or deep seas of 2,000 meters in depth and after examination of the bacillus as in the case of Benton, they reported that some of the strains dissolved chitin most favourably at 0°C. to 4°C.

Of those investigators who are mentioned above, Benton investigated the bacillus chitinovorus most minutely, so my investigation has followed his example mainly. As shown in the above references, the types of bacillus chitinovorus are so abundant in variety that their general biological natures such as shape and size, optimal temperature, sugar fermentation etc. are extremely diverse. The 211 strains which I have isolated and classified into 59 types have a similar chitin destroying characteristic as these isolated by Benecke, Benton and others, but otherwise the strains which I have isolated are not identical. I presume that these strains belong to new types of the bacillus chitinovoros which have never been quoted in the literature of the past. It is believed that perhaps this bacillus is widely distributed everywhere in Japan and that it might be found in more different varieties, therefore the 59 types of bacillus chitinovorus described above will represent only a part of the variety. The nomenclature of the bacillus is in accordance with Bergey's[11] example.

Conclusion

Cultivating 211 strains of bacillus chitinovorus isolated from the mud

or sand of sea beds and fresh water beds in the Kanto, Tohoku and Chubu districts of Japan and the mucous membrane of the stomachs of toads from Tokyo and investigating their general biological natures, I obtained the following results:

1. The 164 strains isolated from mud or sand of sea beds are classified into 40 types in accordance with their general biological natures.
2. The 37 strains isolated from mud or sand of fresh water beds are classified into 16 types.
3. The 10 strains isolated from the mucous membrane of the stomachs of toads from Tokyo are classified into 6 types.
4. As 3 types of the above mentioned have identical characteristics, the 211 strains are classified into 59 types conclusively.
5. Each strain grows at 25°C., but it remains to be proven whether this temperature is optimum or not.
6. All types of the bacillus mentioned above are not identical with any type reported so far.

When completing this article, I came to know that Mr. Akira Saeki had been studying the bacillus since 1949 under the guidance of Dr. Takashi Sekine, Professor of the Tokyo College of Fisheries. I sincerely thank Prof. Dr. Sekine for his kind advice.

This study owes much to the expense for scientific research granted by the Ministry of Education of Japan. I herewith express my thinks. Director O. Miura.

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Congenital Abnormity of Peroxidase Granules— A Case of "Congenital Gigantism of Peroxidase Granules," Preliminary Report

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In Dec. 1950 I found in a 1 year old Japanese infant a striking abnormity of peroxidase granules of all the myeloic leucocytes of blood and marrow. The stain used was the peroxidase raction of Sato and Sekiya.[1] Under this method, the cytoplasm of normal neutrophils is, as is well known, filled with fine peroxidase granules (Cf. Fig. 1), while in this particular case neutrophils have scarcely ever any peroxidase granules except in some limited parts of their cytoplasms where peroxidase granules seem to form exceedingly large masses—"Gigantic Peroxidase Granules" (Cf. Fig. 2). Each granule is spherical, oval or spindle-like in shape, 2–3μ in diameter, intensely blue colored. Each neutrophil contains 1–7 such granules. Such a gigantic peroxidase granule occurs also in monocytes and eosinopils. In these latter such granules are thickly grouped in a place in the cytoplasma. As to myelocytes and myeloblasts, these show similar characteristic abnormity of peroxidase granules only much more conspicuously. Giemsa stain was tried which showed no morphological change corresponding to the abnormal peroxidase granules, though the Giemsa stained picture was in no way normal. Concerning the Giemsa picture and the oxidase stain, I shall report in the near future.

The patient was admitted to the Univ. Hosp. on Dec. 2, 1950, when he had signs of some upper respiratory tract infection, followed by bronchopneumonia. He died on the 17th hospital day, showing hepatosplenomegaly, jaundice, petechiae, melena and pancytopenia. The patient is an offspring of a consanguinous marriage. It is highly probable that the Gigantism of Peroxidase Granules is an evidence of congenital malformations of leucocytes. It is to be noted here that the above mentioned abnormity of peroxidase granules is not limited to a few cells, but to all the myeloic cells in the blood and marrow and I desire to suggest the name of "Congenital Gigantism of Peroxidase Granules" to this congenital disease.

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Fig. 1

Fig. 2

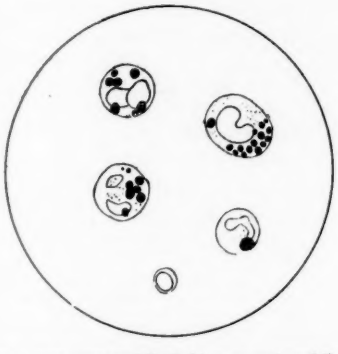

Fig. 1. Normal peroxidase picture of leucocytes: two neutrophils (left), one eosinophil (right upper), one monocyte (righ- lower) and one lymphocyte (left below).
Fig. 2. The "Gigantic Peroxidase Granules" in neutrophils and monocyte (right upper).

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A Chemical Study on the Loach Mucin*

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Chemical study of mucins covering fish body might be of interest not only from the standpoint of fishery but also in the physiological aspect. To begin with, we took the one from loaches (*Misgurnus anguilli caudatus*) as a material, which had been separated by stirring up the fish in distilled water and following precipitation with acid. Paper partition chromatography indicated the presence of glucosamine and galactose as its sugar components, and the color reactions by *p*-dimethylaminobenzaldehyde (Osaki-Turumi method[1]) and Bial orcinol reagent another non-amino acid component, that appeared identical, according to the spectrographic finding, with the acidic compound of Blix[2] from submaxillary mucin. A further investigation is in progress.

Experimental

1 kg. of loaches was stirred up in about 0.5 *l.* of distilled water just covering the fish batch with a glass rod for 10 minutes. The viscid fluid was centrifuged and the supernatant was acidified with 0.1 *N* HCl to a pH of 2.0, whereby most of the mucin was deposited in flakes. The precipitate was washed with a dilute hydrochloric acid solution of the same pH as above, with methanol and with ether in turn and dried in vacuo over P_2O_5. 0.17 g. was the crude product. It was dissolved in a dilute Na_2CO_3 solution of pH 9.0, and after rejecting a small insoluble part, reprecipitated with hydrochloric acid. A greyish powder given dissolved scarcely in plain water but swelled into a gel (even in 100 volumes of water). In dilute alkali it was readily soluble. Batches of the mucin were collected in a similar manner and united together.

Qualitative tests. The results are embodied in Table I.

Paper partition chromatography of sugar components. 20 mg. of the mucin were heated with 5 cc. of 1 *N* H_2SO_4, as sealed in an ampoule, for 3 hrs.

* Read before the 23rd General Meeting of the Japan Biochemical Society, Kobe, April 1951.

TABLE I

Qualitative Assays of the Loach Mucin

Reaction	Result
Biuret	+
Molisch	+
Naphthoresorcinol (for hexuronic acid)	−
Osaki-Turumi test:	
The direct	+
The indirect	+
Rosenthaler (for methylpentose)	−
Bial orcinol-HCl	red coloration
Aniline-acetate (for pentose)	−
Sakaguchi	±
Millon	+ (weak)
Hopkins-Cole	−
Ehrlich diazo	−
Test for P	−
Test for hydrolysable S	−

TABLE II

Composition of the Loach Mucin

Analysis	In per cent	In equivalents per equivalent weight
Total nitrogen*	9.53	
Glucosamine†	17.86	1.00
Galactose‡	9.77	0.54
Acetyl§	9.71	2.31
Reducing power of a hydrolysate as glucose//		
Total reduction	43.06	
Carbohydrate reduction	41.71	
Ash	1.2	

* Micro Kjeldahl, † Hamasato and Akakura method,[5] ‡ Hisamura method,[6] § Suzuki method,[7] // Masamune and Tanabe method[8]; the mucin was hydrolyzed with 1 *N* H_2SO_4 for 3 hrs.

in a boiling water-bath. When cold, the hydrolysate was neutralized with baryta and the centrifuged supernatant was distilled in vacuo to about 0.5 cc., of which 0.01 cc. portions were sampled separately on strips of filter paper. Developing solvent, pyridine-amylalcohol-water (35:35:30 by volume) of Werner and Odin[3]. Indicator, aniline hydrogen phthalate reagent of Partridge.[4] Irrigation was processed in the ascending way.

The chromatograms showed spots corresponding to the positions of glucosamine and galactose.

Quantitative analysis. Table II shows the figures obtained.

Blood group activity (isohemoagglutination inhibition). It was devoid of this potency.

Spectrographic examination. The loach- and submaxillary mucin were examined. The colors developed by the direct and indirect Osaki-Turumi processes were examined in an automatic recording spectrophotometer (Hitachi). The both mucins proved idential with each other in the present respect, giving a dye with absorption maximum at 550 mμ of wave length by the direct reaction, and a dye with two absorption maxima at 540 and 585 mμ, due to acetylhexosamine, by the indirect reaction.

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On Proteins of Gastric Cancer

I. Preparation of Protein Fractions and Some of their Properties, Physical and Chemical*

By

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A large number of gastric cancers were employed and fractioned, by combining the principles described in foregoing articles[1),1a),1b),2)] from this Institute. The present account deals chiefly with separation of the protein fractions and some properties of the products. They are not precluded to have been mixtures of proteins, which contained a minute quantity of carbohydrate or were made up wholly of amino acids, and the group carbohydrate (an amino acid-polysaccharide complex), which had not been perfectly removed due to its tenacious adsorption. But here glucidamins[2a)] are tentatively assumed in place of the mentioned carbohydrate. The corresponding preparations were also prepared from gastric mucosa and examined.

Experiments and Comments

Preparation Procedure

Gastric cancer

Fresh human gastric cancers excised were mechanically freed from adjacent tissue and necrotic matter, and after cutting into small pieces, kept under 5 volumes of alcohol for a duration of time varying from 3 days to 1 year and 8 months. The materials thus obtained were put together according to the blood group of the patients (A and O) and after squeezing and mincing, dried in a Faust apparatus. The number of cancers and the weight of fresh and dried materials were the following.

Blood group	Fresh cancers		Total weight of the dried materials (g.)
	number	total weight (g.)	
A	30	632	98.5
O	30	667	101.0

* The 6th report of Masamune and coworkers' "On Proteins and Amino Acids."

The batches of the dried materials will be referred to as Batches A and O.

1) Batch A

Preliminary fractionation

It was exhausted with boiling alcohol (5 and 3 volumes of abs. alcohol were employed) (Regarding the details, cf. Masukawa[1]). The solutions were united (720 cc.) and set aside for isolation of the blood group lipoid (cf. Masamune *et al.*[3]).

The residue amounting to 88.5 g. was subjected to extraction with changes of water (1000, 900, 900, 800, 800 and 700 cc.). The remainder here, washed with alcohol and dried, weighed 71 g. To the solutions (light-yellow and turbid) were added as much acetic acid (30%) as to raise the pH to 4.5, whereupon marked precipitation occurred, and a little NaCl. The centrifuged supernatant fluids of the earlier solutions were turbid, while those of the later ones were nearly transparent. The centrifugates were washed once with a little water and then three times with abs. alcohol and dried. The successive grayish coarse powders amounted to 0.45, 0.51, 0.86, 0.62, 0.62 and 0.68 g. (in total 3.74 g.—**Ppt. I**).

The supernatant fluids were dialyzed against running water for 24 hours, and distilled in vacuo to about 50 cc., whereby they became much more cloudy. Centrifuged. The centrifugates were washed with water and abs. alcohol and dried. The powders yielded from the 1st to 3rd condensates were 0.23, 0.28 and 0.15 g. respectively (in total 0.66 g.—**Ppt. II**). The centrifugate of the 4th to 6th were very small. The fluids were further condensed to a syrup and precipitated with 5 volumes of abs. alcohol and a little NaCl, followed by repeated washing of the precipitates with abs. alcohol. The amount of the substances dried (light-yellow powders) were 4.43, 0.94, 0.71, 0.56, 0.17 and 0.22 g. (in total 7.03 g.). These were united and agitated with many changes of water (20–40 cc. portions) containing a little NaCl. The insoluble part was washed with abs. alcohol (with addition of a little anhydrous sodium acetate) several times and dried. It amounted to 0.54 g.—**Ins. I.**

The solutions (all slightly turbid) combined were adjusted to pH 9.5 (with Na_2CO_3) and proteins that precipitated were rejected. Following acidulation (with acetic acid) caused extreme turbidity, but gave almost no centrifugate (r. p. m. 3,000). It was then distilled in vacuo to about 10 cc. and 6 volumes of abs. alcohol and a little anhydrous sodium acetate were added. The precipitate was fractioned, after washing with abs. alcohol and drying (6.02 g.), into glacial acetic acid-soluble and -insoluble parts (The substance was kneaded with a little water (2.5 cc.) and an abundant quantity (500 cc.) of 98% acetic acid was added. A little

anhydrous sodium acetate was also used besides). And the insoluble was centrifuged, washed with glacial acetic acid and thoroughly with abs. alcohol and dried—3.61 g. This (a white powder, **Cr. Ch.**) was furnished for separation of the group carbohydrate whose description will be given elsewhere. From the solution—**Fl. I**—were isolated the glacial acetic acid-soluble proteins as below.

Purification

a) Glacial acetic acid-insoluble proteins

α) The proteins precipitable from water by acid

Ppts. I–II and Ins. I (4.9 g. altogether) were agitated together in 100 cc. of water and after addition of the saturated $Ba(OH)_2$ to pH 8.5, stood overnight. To the mixture was again added the baryta solution to pH 9.3, and the abundant precipitate was centrifuged off.[4] The supernatant was acidified to pH 2.0 by means of 3% HCl, and after addition of a little NaCl, subjected to centrifugations (one single centrifugation gave only an extremely turbid supernatant fluid). And the final translucent fluid was dialyzed, concentrated by vacuum distillation to a syrup and mixed with abs. alcohol and a little anhydrous sodium acetate. The deposit was washed with abs. alcohol again and again and dried. A white powder given weighed 1.58 g.—**Ppt. III.** This was combined with Ppts. IV and V below for separation of the fraction which is insoluble in glacial acetic acid but does not precipitate at pH 2.0 from water by acid.

The centrifugate above (the precipitate at pH 2.0) was washed likewise with abs. alcohol and dried. The grayish powder given (0.81 g.) contained phosphorus. It was fractioned by means of glacial acetic acid:—The substance was kneaded with a few drops of water and 3% NaOH. The volume of 98% acetic acid added was 40 cc. The glacial acetic acid-insoluble part, thoroughly washed with the glacial acetic acid and with abs. alcohol (A little anhydrous sodium acetate was added) and dried, amounted to 0.77 g. On it was the sequence of fractionations from the treatment with baryta to that with glacial acetic acid was repeated twice more.[5] A grayish powder amounting to 0.11 g. was yielded—**Prep. I.** Phosphorus was scarcely detected. The fractions recovered from the supernatant fluids of pH 2.0 here will be referred to as **Ppts. IV and V.** They weighed 0.19 and 0.07 g. respectively.

β) The proteins non-precipitable from water by acid

Ppts. III–V were united. This batch (1.84 g.), which also contained phosphorus though little, was taken up in 40 cc. of water and 3% HCl was added to pH 2.0. The precipitate was centrifuged off and washed with three 25 cc. portions of a dilute HCl of pH 2.0, each time followed

by centrifugation.[6] The supernatant fluids (All but the last were turbid) were dialyzed together for 1 day and distilled in vacuo to about 15 cc., then, the condensate was acidified again to pH 2.0 with HCl and precipitated with 20 volumes of a mixture of abs. alcohol and ether (1:1 by volume). The precipitate was washed with the alcohol-ether mixture above and dried. 0.68 g. of a white powder was given here. Another run of removing the part insoluble at pH 2.0 was carried out in a similar manner.[7] The product (a light colorless powder)—**Prep. II**—was free from phosphorus. Crop, 0.51 g.

b) Glacial acetic acid-soluble proteins

To Fl. I were added about 3 volumes of ether. The precipitate, washed with abs. alcohol exhaustively and dried, was fractioned by means of glacial acetic acid over again. Water was not used here at all. The quantity of glacial acetic acid (98%) added was 80 cc. and a little anhydrous sodium acetate was employed besides. After centrifugation, the supernatant fluid (light brown, transparent) and the washings (one 20 cc. and two 10 cc. portions of the glacial acetic acid were used) of the centrifugate[8] were united, precipitated with 4 volumes of ether and centrifuged. By washing the present centrifugate with abs. alcohol containing a little anhydrous sodium acetate and drying, a brownish coarse powder weighing 0.69 g. was given.

By following the procedure in a foregoing account,[1] it was liberated from nucleic acid and basic and acidic proteins precipitable at slight alkalinity and acidity respectively. Namely, the pH of its solution was shifted hither and thither twice with baryta and HCl, followed by removal of barium, concentration of the solution after neutralization with NaOH, and precipitation with alcohol. When thus no contaminants separable in this manner remained anymore, the substance was carried down from the clear solution by means of alcohol—0.54 g. This was light yellow granules and phosphorus-free. Subsequent electrodialysis reduced the quantity of substance to 0.26 g. Finally it was treated over again with glacial acetic acid similar to above, because it was found still partially insoluble in this solvent.[9] The product which was a light-yellow coarse powder—**Prep. III**—amounted to 0.1 g. and was both Molisch- and biuret-positive.

2) Batch O

Fractionation of this batch (101 g.) and purification of its protein fractions were carried out in a manner quite similar to above. The volume of the solutions obtained by extraction with boiling alcohol were 790 cc. altogether. Be the products corresponding to those above represented with the same symbols, Ppts. I and II and Ins. I weighed 3.04,

0.43 and 0.21 g. respectively (in total 3.68 g.) and Ppts. III to V 1.16, 0.28 and 0.09 g. respectively (in total 1.52 g.). Cr. Ch. 3.24 g. Of the purified fractions, which were insoluble in glacial acetic acid, the one precipitable by acid (Prep. I) was 0.15 g. and the one non-precipitable by acid (Prep. II) 0.32 g., and the glacial acetic acid-soluble (Prep. III) 0.18 g. These fractions resembled the corresponding fractions of Batch A in appearance as well as the result of biuret and phosphorus test.

Normal human gastric mucosa

The mucosa was teared from the whole or a part of a human stomach and after freeing from mucus, cut into small pieces, which were preserved in 5 volumes of alcohol for a various duration of time from 13 days to 17 months. The materials thus obtained from stomachs were also divided according to the blood group of the persons, squeezed and dried—Batches A and O. Details are tabulated as below:

Blood group	Fresh materials		Total weight of the dreid materials (g.)
	number	weight (g.)	
A	19	1,254	224
O	14	927	174

The fractionation procedure was strictly same as above.

1) Batch A

Batch A amounted to 224 g. After treatment with boiling abs. alcohol, it weighed 208 g. The light brown alcoholic solutions amounted to 1800 cc. in all. The fractions corresponding to Ppts. I and II and Ins. I above were 4.81, 0.61 and 1.13 g. respectively (in total, 6.55 g.) and those corresponding to Ppts. III to V 1.60, 0.21 and 0.04 g. (in total, 1.85 g.). The crude fraction (Cr. Ch.) set aside for separating the group carbohydrate was 1.39 g. The purified proteins, insoluble in glacial acetic acid and precipitable from water by acid, 0.11 g.; those, insoluble in glacial acetic acid and unprecipitable from water by acid, 0.35 g.; the glacial acetic acid-soluble, 0.11 g.

2) Batch O

Batch O, 174 g. The hot-alcoholic solutions, 1260 cc. The crude fractions of the proteins which were insoluble in glacial acetic acid, 0.75, 0.75 and 0.45 g. respectively (in total 1.95 g.). The material containing the group carbohydrate (Cr. Ch.), 0.68 g. The fractions of glacial acetic acid-insoluble proteins could not be thoroughly purified due to shortage of material. The purified glacial acetic acid-soluble amounted only to 57 mg.

The fractions, crude and purified, from the both batches of the mucosa had appearance like the corresponding fractions from gastric cancers and also gave same consequences in qualitative tests.

Physical and Chemical Properties of the Products

The corresponding protein fractions from the different sources resembled one another in solubility, precipitability, test-tube assays and quantitative analyses.

1) Solubility and precipitability

Prep.-I's dissolved in water on alkalization and half saturation with $(NH_4)_2SO_4$ gave rise to remarkable precipitation.

Prep.-II's dissolved also in water, forming a clear solution, on faint alkalization. Trichloroacetic acid and $(NH_4)_2SO_4$ (even at half saturation) gave rise to a precipitate from its solution, but picric acid could not. The solution became turbid on acidification (turbidity maxima at pH's 4.0 and 2.4), but no precipitation occurred.

Prep.-III's. Their solutions behaved similar to Prep.-II's towards trichloroacetic acid and $(NH_4)_2SO_4$.

2) Chemical qualitative tests

Prep.-I's. Biuret-, Millon-, Sakaguchi-, reduced sulfur-, Hopkins-Cole- and Molisch-test positive. The positive Goldschmiedt and Neuberg-Saneyoshi reaction were given, although aniline acetate test resulted only in faint positive. Rosenthaler weak. The indirect Osaki-Turumi reaction was shown but not the direct.

Prep.-II's. The biuret test and the assays for amino acids resulted similar to above. Molisch and the reactions of hexuronic acid and methylpentose occurred more intensively than in Prep.-I's. The direct Osaki-Turumi reaction was given, though faint, besides the positive indirect reaction.

Prep.-III's. The tests resulted similar as in Prep.-I's but that more vivid Rosenthaler was given.

3) Quantitative composition

Analysis was made by our usual procedures. The figures obtained are embodied in Tables I–III, which demonstrate that the corresponding fractions from gastric cancers and the mucosa were generally similar in so far as the rough composition concerns. Prep.-III's from gastric mucosa were richer in carbohydrate than those from gastric cancers, but such a divergency as was found may happen between mixture preparations

of this kind even if separated from parts of one and the same batch of a tissue pulp.

4) Optical rotation

As is indicated in Tables II and III, Prep.-II's and Prep.-III's of cancer

TABLE I

Composition of Prep.-I's (the protein fraction of gastric cancer and gastric mucosa insoluble in glacial acetic acid and precipitable from water by acid)

Analysis	The protein fraction		
	of gastric cancer		of normal gastric mucosa from Group A persons
	from Group A persons	from Group O persons	
N*	12.3	13.0	14.2
Hexosamines†	7.9	6.1	5.4
Non-amino sugars as galactose			
Orcinol reaction	8.8	8.7	8.1
Indole r.	15.2	16.5	16.8
Diphenylamine r. I.	14.7	12.3	10.8
Ash	11.1	11.7	12.6

* Micro Kjeldahl. † Hamasato-Akakura method

TABLE II

Composition and Optical Rotation of Prep.-II's (the protein fraction of gastric cancer and normal gastric mucosa insoluble in glacial acetic acid and unprecipitable from water by acid)

Analysis and rotation	The protein fraction		
	of gastric cancer		of normal gastric mucosa from Group A persons
	from Group A persons	from Group O persons	
Composition (Figures express percentages.)			
N	9.5	9.7	10.3
Hexosamines	20.0	21.2	20.8
Non-amino sugars as galactose			
Orcinol reaction	20.1	21.0	18.0
Indole r.	27.1	28.6	27.8
Diphenylamine r. I.	35.2	36.2	25.1
Ash	4.2	3.9	2.7
Optical rotation			
$[\alpha]^D$	−26.8° (11°C)	−25.1° (11°C)	−41.0° (11°C)

TABLE III

Composition and Optical Rotation of Prep.-III's (the protein fraction, soluble in glacial acetic acid, of gastric cancer and normal gastric mucosa)

Analysis and rotation	The protein fraction			
	of gastric cancer		of normal gastric mucosa	
	from Group A persons	from Group O persons	from Group A persons	from Group O persons
Composition (Figures express percentages; those in parentheses are the calculated on an ash-free basis)				
N	10.8 (13.1)	10.7 (12.8)	9.7 (11.4)	10.5 (11.8)
Hexosamines	10.6 (12.9)	10.6 (12.7)	15.9 (18.6)	18.2 (20.4)
Non-amino sugars as galactose Orcinol reaction	14.8 (18.0)	13.8 (16.5)	18.4 (21.5)	21.0 (23.5)
Indole r.	20.0 (24.3)	20.0 (24.0)	22.8 (26.7)	29.3 (32.8)
Diphenylamine r. I.	22.4 (27.2)	25.7 (30.8)	27.5 (32.2)	30.2 (33.9)
Ash	17.6	16.6	14.6	10.7
Optical rotation				
$[\alpha]_D$	−50.9° (11°C)	−51.8° (12°C)	−40.6° (12.5°C)	−55.8° (13°C)

had rotations almost equal between the Groups A and O within each pair of the preparations. Moreover the rotations of the latter preparations (the Prep.-III's) lay between those of the corresponding mucosa preparations.[10] Prep. II could not be prepared from the gastric mucosa of Group O persons, but the rotation of that from gastric mucosa of Group A persons suggests that a smiliar relation also holds within the cancer and mucosa Prep.-II's. These facts reminds one of Oh-Uti's finding[11b] on the rotations of the Group A and Group O carbohydrates of gastric cancer and gastric mucosa.

Summary

1. Three protein-fractions (the fraction which is insoluble in glacial acetic acid and precipitates from water at pH 2.0 (I), the one insoluble in glacial acetic acid and non-precipitable at any pH from water (II), and the glacial acetic acid-soluble (III)) were separated from a gastric cancer of persons belonging to Group A and a gastric cancer of Group

O persons. Also the corresponding preparations were prepared from human gastric mucosa.

2. The solubilities, qualitative reactions and quantiativea nalyses resembled each other between the corresponding fractions of the gastric cancer and mucosa.

The rotation of a pair of preparations from cancers of persons belonging to Group A and from those of persons belonging to Group O were coincident with each other and lay between the rotations of the corresponding preparations from gastric mucosa.

Through the Grant Committee for the Scientific Researches was given a grant from the Education Department, which enabled us to carry out this investigation. H. Masamune.

References and Notes

1) Masukawa, Tohoku J. Exp. Med., 1951, **54**, 227; Matuda, *ibid.*, 1951, **53**, 333.

1a) Satoh, *ibid.*, 1949, **51**, 275.

1b) Oh-Uti, K., *ibid.*, 1949, **51**, 297.

2) Aikawa, *ibid.*, 1949, **51**, 367.

2a) Masamune, *ibid.*, 1949, **50**, 107; Masamune, Yosizawa & Maki, *ibid.*, 1951, **53**, 237 (footnote).

3) Masamune, Siozima & Masukawa, *ibid.*, 1951, **54**, 327.

4) The centrifugate was washed with a dilute baryta solution of pH 9.3 and then with abs. alcohol (A little anhydrous sodium acetate was employed besides) and dried—Ppt. A. It amounted to 2.1 g.

5) The portions precipitated by baryta—Ppts. A′ and A″—weighed 0.3 g. and 70 mg., when they were dried after washing with a dilute baryta of pH 9.0 and alcohol.

6) The centrifugate was then washed well with alcohol and dried—Ppt. B. This weighed 0.8 g.

7) The centrifugate here, washed and dried, amounted to 155 mg.—Ppt. B′.

8) This was further washed with abs. alcohol several times and dried—Ppt. C. It amounted to 0.35 g.

9) The insoluble part, centrifuged, was washed once with glacial acetic acid and many times with abs. alcohol and dried. A yellowish powder weighing 86 mg. was given—Ppt. C′.

10) Judging from the analyses, Prep.-III's from the mucosa and those from the cancers don't seem to strictly correspond to each other regarding the quantiative ratios of the proteins composing the respective substances, and hence their rotations must be compared mutually with some reservation.

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On Proteins of Gastric Cancer

II. Further Phsyical and Chemical Properties of the Protein Fractions*

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(Received for publication, February 3, 1953)

In continuation of the former report,[1] the results of electrophoresis of the protein fractions of gastric cancers and gastric mucosa, paper partition chromatography of their components and else will be described here.

Experiments and Comments

Electrophoresis

Preps. II's and III's obtained from the cancers and mucosa in the former report[1] which were the fractions insoluble in glacial acetic acid and non-precipitable from water by acid and the ones soluble in glacial

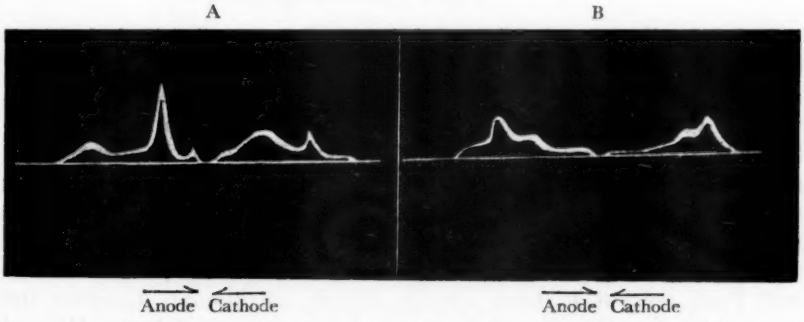

Fig. 1. Electrophoretic pattern of Preps. II's. A: pattern of a 1.2% solution of Prep. II from gastric cancer of persons belonging to Group A; 15°C; current 8 ma; exposure 30 min. B: pattern of a 1% solution of Prep. II from gastric mucosa of persons belonging to the same blood group as above; 14°C; current 8 ma; exposure 45 min. Ascending limbs to the left.

* The 7th Report of Masamune and coworkers' "On Proteins and Amino Acids."

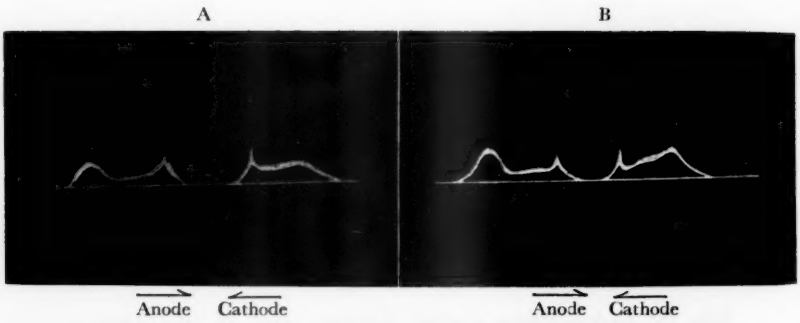

Fig. 2. Electrophoretic pattern of a 36/27% solution of Preps. III's. A: pattern of Prep. III from gastric cancer of persons belonging to Group O; 14°C; current 9 ma; exposure 30 min. B: pattern of Prep. III from gastric mucosa of persons belonging to Group A; 15°C; current 9 ma; exposure 30 min. Ascending limbs to the left.

acetic acid respectively, were examined in a Tiselius electrophoresis apparatus, employing a phosphate buffer of pH 7.8 and I 0.29 (Figs. 1–2).

The pattern of the pair of Preps. III's are similar to each other, showing two similar corresponding boundaries. But that of the pair of Preps. II's both with three boundaries are dissimilar, the relative velocity of the chief component among the others in one preparation being not in accord with that in the other preparation.

Paper Chromatography

Amino acids. Preps. II's and III's were examined. Hydrolysis was effected by heating at 100°C with 6 *N* HCl (under reflux). The hydrolysates were removed from HCl by repeated vacuum-distillations with water. The pH of the solutions sampled for irrigation were 2.0–3.0. Solvents: 1) butanol-acetic acid-water (4:1:5)[2] and 2) phenol-water (4:1). Two-dimension chromatography was processed by using these solvents in the arranged or inverted order. Indicator, 0.2% ninhydrin in butanol. Besides, chromatograms run in parallel by Solvent 1 alone were developed by means of not only ninhydrin but also platinum iodide and Pauli reagent to more strictly identify methionine, histidine and tyrosine (S. Fig. 4). Figs. 3–5 are the chromatograms of Preps. II's and Figs. 6–7 those of Preps. III's. The former Figs. show that Prep. II of gastric cancers (Group A) and that of gastric mucosa (Group A) both contained aspartic and glutamic acid, serine, glycine, threonine, alanine, methionine and/or valine, leucine and/or isoleucine, phenylalanine, tyrosine, lysine, histidine, arginine and probable underglutamic acid. All the chromatograms showed one unidentified spot X (a probable peptide)

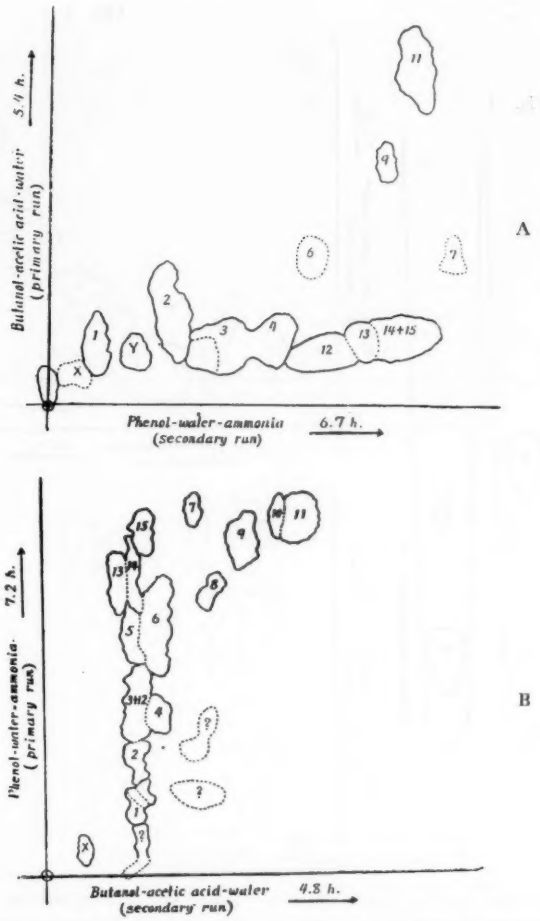
Butanol-acetic acid-water (primary run) 5.4 h.
Phenol-water-ammonia (secondary run) 6.7 h.
A
Phenol-water-ammonia (primary run) 7.2 h.
Butanol-acetic acid-water (secondary run) 4.8 h.
B

Fig. 3. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. II from a gastric cancer of persons belonging to Group A. 28 mg. of the preparation were heated with 5 cc. of 6 *N* HCl for 27 2/3 hrs. The solution prepared for irrigation (pH 2.8) was 0.6 cc., of which 0.03 and 0.02 cc. portions were sampled in A and B respectively. Temperature of test 19°±1°C. Indicator ninhydrin.

1: aspartic acid, 2: glutamic acid, 3: serine, 4: glycine, 5: threonine, 6: alanine, 7: proline, 8: tyrosine, 9: valine and/or methionine, 10: phenylalanine, 11: leucine and/or isoleucine, 12: hexosamine, 13: lysine, 14: histidine, 15: arginine, 16: hydroxyproline (?), X, Y, Z: not exactly identified. (Some of the spots are not seen here).

Fig. 4

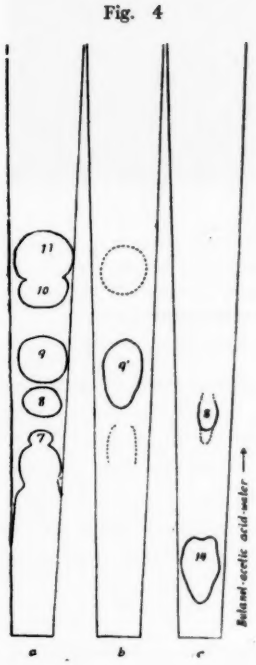

Fig. 5

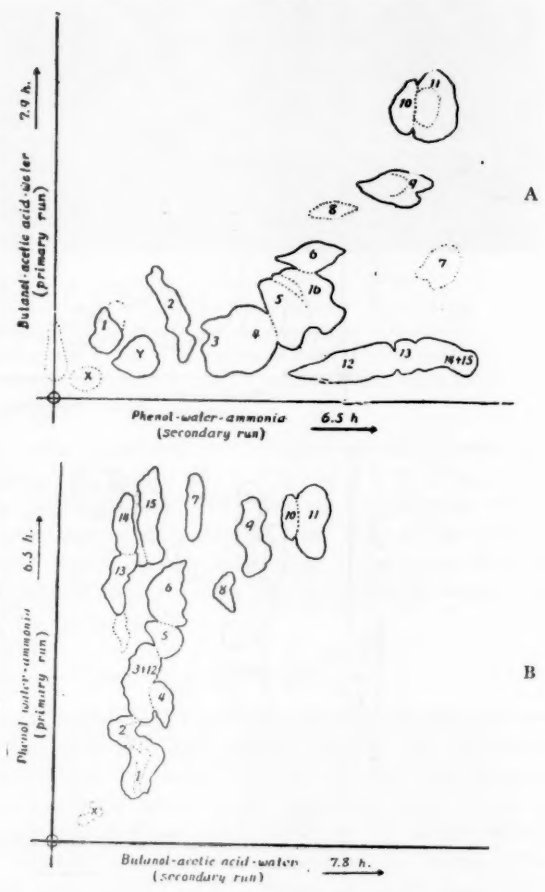

Fig. 4. Diagrammatical reproduction of butanol-acetic acid-water chromatograms of the hydrolysate solution used for the chromatogram in Fig. 3. 0.05 cc. portions were sampled. Paper strips 30 cm. × 2.2 cm. to 4.4 cm. (trapezoids). Temperature of test, 19°±1°C. a, b and c were developed with ninhydrin, platinum iodide and Pauli reagent respectively.

Ciphers express the same as in Fig. 3.

Fig. 5. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram and a chromatogram irrigated in the inverted order of Prep. II from normal gastric mucosa of persons belonging to Group A. 33.5 mg. of the preparation were heated with 6 cc. of 6 *N* HCl for 29 2/3 hrs. The solution prepared for irrigation (pH 2.0) was 0.8 cc., of which 0.02 cc. and 0.03 cc. portions were sampled in A and B. Temperature of test 19°±1°C. Indicator, ninhydrin.

The ciphers and characters signify the same as in Fig. 3.

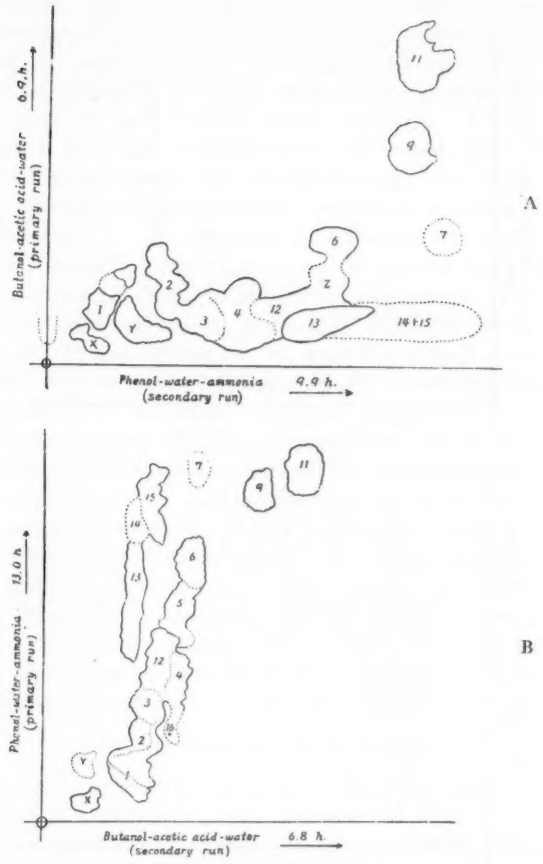
Butanol-acetic acid-water (primary run) 0.9 h.
Phenol-water-ammonia (secondary run) 9.9 h.
A
Phenol-water-ammonia (primary run) 13.0 h.
Butanol-acetic acid-water (secondary run) 6.8 h.
B

Fig. 6. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. III from a gastric cancer of persons belonging to Group O. 30 mg. of the preparation were heated with 6 cc. of 6 *N* HCl for 22 3/4 hrs. The solution prepared for irrigation (pH 2.6) was 0.6 cc., of which 0.01 and 0.012 cc. portions were sampled in A and B respectively. Temperature of test 19°±1°C. Indicator, ninhydrin.

The ciphers and characters signify the same as in Fig. 3.

besides. In Fig. 3 there are seen three spots marked with ?, which, however, are difficult to judge if they represent any amino acids or are to be ascribed to technical fault. Spot 16 in Fig. 5, a reproduction of the chromatogram of Prep. II of the mucosa, falls approximately on the

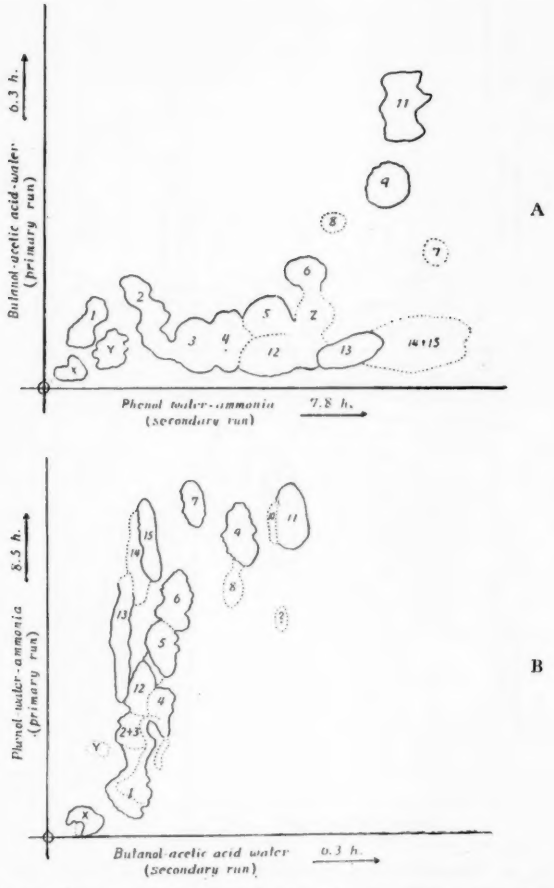

Fig. 7. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. III of gastric mucosa of persons belonging to Group A. 30 mg. of the preparation were heated with 6 cc. of 6 *N* HCl for 22 3/4 hrs. The solution prepared for irrigation (pH 2.6) was 0.6 cc., of which 0.01 and 0.012 cc. portions were sampled in A and B respectively. Temperature of test 19°±1°C. Indicator, ninhydrin.

Ciphers and characters signify the same as in Fig. 3.

position of hydroxyproline, whose corresponding spot was not detected in the chromatograms of the corresponding preparation of the gastric cancer from persons of the same blood group (Fig. 3). Figs. 6–7 demon-

strate that Preps. III's from gastric cancers (Group O) and mucosa (Group A) contained aspartic and glutamic acid, serine, glycine, threonine, alanine, proline, valine and/or methionine, leucine and/or isoleucine, lysine, histidine, arginine, probable underglutamic acid (Y), a probable peptide (X) and an unidentified amino acid (Z). The hydrolysate of the preparation from the mucosa gave additional spots corresponding to positions of phenylalanine and tyrosine though very weak and another unidentified spot which was seemingly produced by technical fault (the spot with ? in Fig. 7). By sampling a larger amount of the hydrolysate of the cancer substance and irrigation in the second way, phenylalanine could be demonstrated, but tyrosine could not at all. The cancer and mucosa preparations employed here had been obtained from tissue materials from persons belonging to different blood groups. But the possibility that the tyrosine deficiency of the cancer substance may be due to its being an O substance might be precluded.

In the corresponding chromatograms of any pair of the preparations from the cancers and mucosa, some of the corresponding amino acid-spots were so divergent in colour strength that their different contents must be assumed according to the source of the substances.

Sugars. Prep. III of Group A gastric cancer and mucosa were formerly examined and reported[3] to contain chondrosamine, glucosamine, galactose and fucose. Processed in a similar manner (kindly tested by Dr. Yosizawa), Prep.-II's from the gastric cancer of Group A and gastric mucosa of the same blood group showed spots at positions of the sugars above, and, in addition, a weak spot of mannose (Fig. 8).

Optical Rotation of Hydrolysates

Prep. II from the gastric cancer of Group A persons was hydrolyzed as for amino acid chromatography (duration of hydrolysis 13 2/3 hrs.). The hydrolysate was freed from HCl as far as possible by repeated vacuum-distillations with water. The final still residue was taken up in a little water and filtered, and the filtrate and washing were evaporated together on a boiling water-bath. The remainder here was dried to constant and 7.1 mg. of it were taken up in 2.1 cc. of water (conc. 0.338%, pH 4.3). The rotation was measured after 24 hrs. from dissolution:

$$[\alpha]_D^{\gamma} = \frac{-0.183 \times 100}{0.338 \times 1} = -54.1°.$$

The corresponding preparation from the gastric mucosa of persons of the same blood group was hydrolyzed with HCl and the insoluble humin eliminated. 17.6 mg. of the final evaporated residue were yielded from 29.6 mg. of the mucosa substance, and a 0.562% solution (pH 4.2) was prepared to determine the rotation after 24 hrs.:

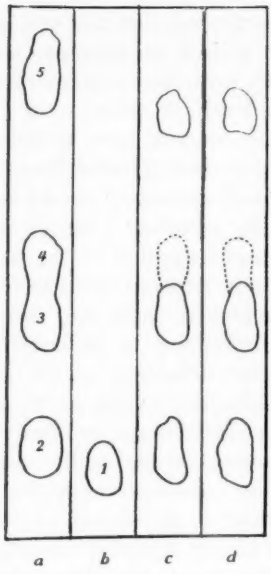

Fig. 8. Diagrammatical reproduction of a *n*-butylacetate-acetic acid-water (3:2:1) chromatogram of Preps. II's from the gastric cancer of Group A persons and gastric mucosa of persons of the same blood group. 50 mg. of each of the substances were hydrolyzed in an ampoule with 5 cc. of 1 *N* H_2SO_4 by heating at 100°C for 5 hrs. The solution of the hydrolysates prepared for irrigation were both 0.5 cc., of each of which a 0.015 cc. portion was sampled. Duration and temperature of test, about 15 hrs. and 20°±1°C. Indicator, aniline hydrogen phthalate of Partridge.

a and b: standards. c: Prep. II from gastric cancers. d: Prep. II from gastric mucosa. 1: chondrosamine sulfate, 2: glucosamine sulfate, 3: galactose, 4: mannose, 5: rhamnose.

$$[\alpha]_D^{\prime 0}=\frac{-0.109\times 100}{0.562\times 1}=-19.4^{\circ}.$$

Thus the rotation of the hydrolysates were found incoincident, in harmony with the consequences of chromatography suggesting the different amino acid contents of the preparations.

Summary

The electrophoretic patterns of Prep. III of the gastric cancers and mucosa resembled each other. But, those of Preps. II's differed from each other, although they both demonstrated three boundaries. Concerning the amino acid composition, Preps. II's and III's were somewhat different qualitatively as well as quantitatively from each other in

respective pair of preparations from the two sources. The optical rotations of a hydrolysate of the Preps. II's supported this fact. Combined with the analytical results in the former report, the present findings indicate that the corresponding proteins of gastric cancer and the mucosa are not identical despite of their resemblance in rough composition.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department which enabled us to carry out this investigation.
H. Masamune.

References and Notes

1) Masukawa, Tohoku J. Exp. Med., this volume, p. 251.
2) The upper layer of the equilibrated mixture was taken for the purpose of irrigation.
3) Masamune, Yosizawa, Oh-Uti, Matuda & Masukawa, Tohoku J. Exp. Med., 1952, **56**, 37.

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On Proteins of Gastric Cancer

III. Biological Properties of the Protein Fractions*

By

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The protein fractions, Preps. I, II and III, of gastric cancers and human gastric mucosa in the foregoing reports[1][2] were further examined regarding their biological properties such as hemoagglutination inhibition, precipitin reaction with the immune sera, hemolytic activity and skin reaction.

Experiments and Comments

Preps. I and II were the fractions both insoluble in glacial acetic acid but precipitable and unprecipitable respectibly by acid, and Prep. III the fraction soluble in glacial acetic acid (S. the 1st report).

Anti-hemoagglutinative force. Inhibition of hemoagglutination was examined by our usual procedure (Cf. Oh-Uti[3]). The anti-O serum, prepared by injection of O erythrocytes to a goat, was diluted 10 times and absorbed with Be erythrocytes. Thus treated, it agglutinated O erythrocytes at a dilution of $1:4\times10^2$ but Be erythrocytes only faintly. The consequences of the assay are shown in Tables I–II. The fractions of Group A cancers were all specifically group active but were weaker than the corresponding fractions of Group A mucosa. Prep. III from Group O cancer was also less anti-agglutinative than that from the mucosa of the same blood group.

Precipitin reaction with the rabbit antisera of the cancer (Group B), gastric mucosa (Group O) and placenta.[4] The rabbit antisera, kindly supplied by Dr. Kobayasi,[5] were absorbed with normal human serum of Group AB (2 drops per cc.) prior to the assay. The results are illustrated in Table III.

Preps. I's from the cancers and gastric mucosa reacted to equal extents with either of the anti-cancer and anti-mucosa sera, although

* The 8th report of Masamune and coworkers' "On Proteins and Amino Acids."

Table I

Agglutination of A and B Erythrocytes by Normal Sera, treated with the Cancer and Mucosa Fractions

B and A sera had titre 320 and 160 and diluted 20 and 10 times respectively. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., #: strong aggl.

Fraction for absorption		Blood group of red cells	Dilution of the fractions (1:)																			
			10^2	2×10^2	4×10^2	5×10^2	8×10^2	10^3	2×10^3	4×10^3	5×10^3	8×10^3	10^4	1.6×10^4	2×10^4	4×10^4	8×10^4	10^5	2×10^5	4×10^5	8×10^5	10^6	2×10^6
Group A cancer	Prep. I	A	−	−	+		+	+	+	++		#	#					#					
		B	−	+	+		++	++	#	#		#	#										
	Prep. II	A	−	−	−		−	−	−	±		+	++					#					
		B	−	−	±		±	+	+	++		++	++					#					
	Prep. III	A	−	−	−		±	+	+	+		+											
		B	+	+	+		+	+	+	+		+											
Group A mucosa	Prep. I	A						−					−		−	−	±	++	#	#	#	#	
		B						#					#		#	#	#	#	#	#	#	#	
	Prep. II	A						−					−					−	−	−	−	±	+
		B						#					#					#	#	#	#	#	#
	Prep. III	A				−		−	±	+	+	+		+	+	+							
		B				#		#	#	#	#	#		#	#	#							

Table II

Agglutination of O Erythrocytes by the Diluted and Absorbed Immune Serum, after Treatment with the Cancer and Mucosa Fractions

−: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., #: strong aggl.

Fractions for absorption		Dilution of the fractions (1:)													
		10^2	2×10^2	4×10^2	8×10^2	10^3	1.6×10^3	2×10^3	3.2×10^3	4×10^3	6.4×10^3	8×10^3	10^4	1.3×10^4	2×10^4	2.6×10^4
Group O cancer	Prep. I		−	−	−		+		++		++					
	Prep. II	−	−	−	−	−		−		−		++	++		#	
	Prep. III	−	±	+	+	++		#		#				·		
Group O mucosa	Prep. III			−	−		−		±		+			#		#

TABLE III

Precipitin Reaction (Ring Test) of the Preparations from Cancers and Gastric Mucosa with the Immune Rabbit Sera absorbed with Normal Human Serum

−: no precipitation, ±: faint precip., +: definite precip.

Prep.	Source of Preps.	Dilution of the preparations (1 :)														
		50	10^2	2×10^2	4×10^2	8×10^2	10^3	2×10^3	3×10^3	4×10^3	5×10^3	6×10^3	7×10^3	8×10^3	9×10^3	10^4
	Anti-cancer serum															
	Cancer (O)		+	+	+	+	+	+	+	+	+	±	−	−	−	
	Gastric mucosa (A)		+	+	+	+	+	+	+	+	+	+	−	−	−	
	Anti-mucosa serum															
I's*	Cancer (O)		+	+	+	+	+	+	−	−	−	−	−	−	−	
	Gastric mucosa (A)		+	+	+	+	+	+	+	−	−	−	−	−	−	
	Anti-placenta serum															
	Cancer (O)		−	−	−	−										
	Gastric mucosa (A)		−	−	−	−										
	Anti-cancer serum															
	Cancer (A)	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	Gastric mucosa (A)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	Anti-mucosa serum															
II's*	Cancer (A)	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
	Gastric mucosa (A)	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	Anti-placenta serum															
	Cancer (A)	−	−	−	−	−	−									
	Gastric mucosa (A)	−	−	−	−	−	−									
	Anti-cancer serum															
	Cancer (O)	+	±	−	−	−										
	Gastric mucosa (A)	±	±	−	−	−										
	Anti-mucosa serum															
III's	Cancer (O)	−	−	−	−	−										
	Gastric mucosa (A)	±	−	−	−	−										
	Anti-placenta serum															
	Cancer (O)	−	−	−	−	−										
	Gastric mucosa (A)	±	−	−	−	−										

* The substance was dissolved in physiological saline by the aid of a small quantity of NaOH.

these sera showed marked discrepancy of potency on their side. Contrariwise, Prep. II from the cancers (Group A) was less reactive to those sera than the corresponding preparation from stomach mucosa. However, the former substance gave a precipitate with the anti-cancer serum even after complete absorption with the latter. Namely, the less reactive one of the pair of the preparations did not have the perfectly same specificity in reaction that the other had. That these preparations gave different electrophoretic patterns (the 2nd report) may have some connection with this finding. In this regard a detailed study will be reported later from this Institute. The antibody of Prep.-III's in the antisera were too minute for revealing a definite fact, and, moreover, the results obtained with the anti-placenta serum showed absence of reactive antidodies.

Hemolysis. A 0.5% solution of Preps. II's and III's from the gastric cancers and mucosa (both of persons of Group A) were prepared, and to 0.5 cc. each of the original and progressively diluted solutions were added 2 drops of a 3% erythrocyte (Group O) suspension. The mixtures were stood in a 37°C thermostat for 3 hrs., shaking at intervals of 30 minutes. Table IV shows the results. In the controls, in which the solutions were substituted with physiological saline, no hemolysis occurred.

The fractions from gastric cancers looked to be entirely or almost inert. Those from normal gastric mucosa effected hemolysis but as slightly as may be ascribed to a contaminant.

Cancer skin reaction. A 0.56% solution of the preparations were prepared, employing sterile physiological saline, with slight alkalization for dissolution of the substance and subsequent neutralization when necessary, and a 1/9 volume of 5% phenol added. The original solutions thus obtained were diluted appropriately with the saline and phenol solution before use. After 5 hours from the intracutaneous injection of a 0.2 cc.

TABLE IV

Occurrence of Hemolysis by Preps. II's and III's

−: no hemolysis, ±: faint hemol., +: partial hemol., ++: half hemol., ##: nearly complete hemol.

Prep.	Source of Preps.	Dilution of the preparations (1 :)							
		2×10^2	4×10^2	8×10^2	2×10^3	4×10^3	8×10^3	2×10^4	4×10^4
II's*	Cancer (A)	−	−	−	−	−	−	−	−
	Gastric mucosa (A)	+	±	−	−	−	−	−	−
III's	Cancer (A)	±	−	−	−	−	−	−	−
	Gastric mucosa (A)	##	+	−	−	−	−	−	−

* The substance was dissolved in physiol. saline by the aid of a minute quantity of NaOH.

Fig. 1

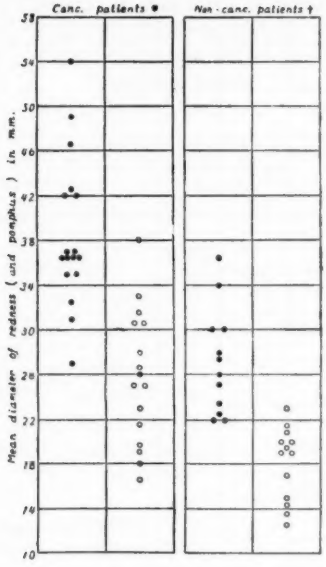

Fig. 2

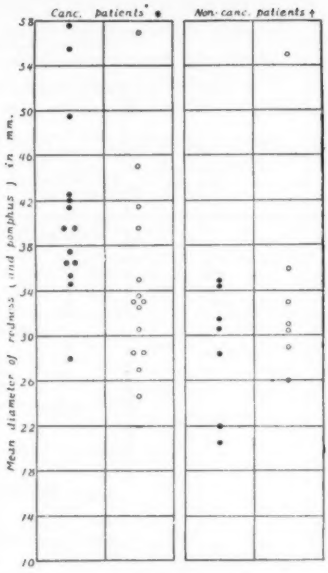

Fig. 1. Showing the results of the skin test with Prep.-I's from gastric cancers (Group O) and gastric mucosa (Group A) (both diluted to 0.1%).
• the cancer substance. ○ the mucosa substance. * carcinoma of oesophagus (1), stomach (3), uterus (7), pancreas (2), tongue (1), biliary ducts (1), and liver (1), .and osteosarcoma (1). † haemangioma (1), appendicitis (1), enteroptosis (1) and gastric or duodenal ulcer (9).
Judgement: the cancer substance, positive; the mucosa substance, positive.

Fig. 2. Showing the results of the skin test with Prep.-II's from gastric cancers and gastric mucosa both of Group A. Dilution of the substances 0.1%.
• the cancer substance. ○ the mucosa substance. * carcinoma of stomach (7), liver (2), pancreas (2), rectum (1), oesophagus (1) and biliary ducts (1). † gastric ulcer (2), fracture (1), tuberculosis of kidney (1), tuberculous pleuritis (1), haemorrhoids (1) and chronic pancreatitis (1).
Judgement: the cancer substance, positive; the mucosa substance, positive (not distinct).

portion on the inner side of forearm, the longest and shortest diameters of the redness and pomphus were measured and their total sum divided with 4 (When no pomphus appeared, the sum of diameters of the redness was divided with 2). Figs. 1–3 show the average diameters thus obtained in cancerous and non-cancerous patients.

It may be asserted therefrom that the preparations from gastric cancers give generally a larger redness and pomphus in the former group of

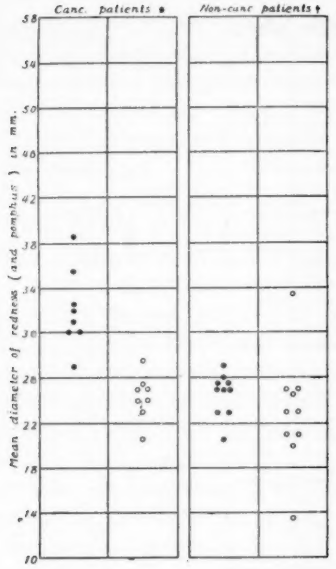

Fig. 3. Showing the results of the skin test with Prep.-III's from gastric cancers of Group O and gastric mucosa of Group A (both diluted to 0.1%).
• the cancer substance. ◦ the mucosa substance. * carcinoma of stomach (4), mamma (1), rectum (1) and submaxillary gland (2). † gastric or duodenal ulcer (10).
Judgement: the cancer substance, positive; the mucosa substance, negative.

patients than in the latter. The preparations from gastric mucosa also showed some such tendency but gave smaller spots.

Effect of tryptic digestion on the precipitin and cancer skin reaction of the preparations. 1) Digestion. A weighed amount (15–30 mg.) of a preparation and an equal amount or more of trypsin (Kahlbaum) were taken up in water (50 to 250 volumes) and after addition of a 1% Na_2CO_3 solution to pH 9.0 and two drops of toluene, placed in an incubator at 37°C. pH of the mixture was readjusted every 12 hrs. to the original, until it ceased to change. Then the digestate was neutralized with a 1% HCl solution and heated, as stoppered with cotton, in a boiling water-bath for 10 minutes.

2) Precipitin reaction of the digestates with immune rabbit sera (anti-Group B cancer and anti-Group O mucosa serum) prepared as the ones used above. For the present purpose, Preps. I's from Group O

TABLE V

Precipitin Reaction of the Digestate of Preps. I's from Group O Gastric Cancers and Group A Mucosa together with that of the Undigested Materials with an Anti-cancer and an Anti-mucosa Rabbit Sera absorbed with Human Serum (Group AB)

The mixtures incubated for digestion, 10 mg. Prep. I and 10 mg. trypsin in 1 cc. of water; duration of incubation, 14 days. −: no precipitin reaction, +: occurrence of the reaction.

Antigen from	Dilution of antigens (1 :)									
	10^2	2×10^2	4×10^2	8×10^2	10^3	2×10^3	3×10^3	4×10^3	....	10^4
Anti-cancer serum										
Group O cancer digested	+	+	+	+	+	−	−	−	−	−
Group O cancer undigest.	+	+	+	+	+	+	−	−	−	−
Group A mucosa digested	+	+	+	+	+	+	−	−	−	−
Group A mucosa undigest.	+	+	+	+	+	+	−	−	−	−
Anti-mucosa serum										
Group O cancer digested	+	+	+	+	−	−	−	−	−	−
Group O cancer undigest.	+	+	+	+	−	−	−	−	−	−
Group A mucosa digested	+	+	+	+	−	−	−	−	−	−
Group A mucosa undigest.	+	+	+	+	+	−	−	−	−	−

TABLE VI

Precipitin Reaction of the Digestate of Preps. II's from Group A Gastric Cancer and Group A Mucosa together with that of the Undigested Materials with the Absorbed Anti-cancer and Anti-mucosa Sera in Table V

The mixture incubated for digestion, 15 mg. Prep. II +15 mg. trypsin in 1.5 cc. water; duration of incubation, 18 days. −: no precipitin reaction, +: occurrence of the reaction.

Antigen from	Dilution of antigens (1 :)											
	10^2	....	8×10^2	10^3	2×10^3	3×10^3	4×10^3	....	8×10^3	9×10^3	10^4	1.2×10^4
Anti-cancer serum												
Group A cancer digested	+	+	+	+	+	+	−	−	−	−	−	−
Group A cancer undigest.	+	+	+	+	+	+	−	−	−	−	−	−
Group A mucosa digested	+	+	+	+	+	+	+	+	+	+	−	−
Group A mucosa undigest.	+	+	+	+	+	+	+	+	+	+	+	−
Anti-mucosa serum												
Group A cancer digested	−	−	−	−	−	−	−	−	−	−	−	−
Group A cancer undigest.	+	+	+	−	−	−	−	−	−	−	−	−
Group A mucosa digested	+	+	+	+	+	+	+	+	+	−	−	−
Group A mucosa undigest.	+	+	+	+	+	+	+	+	+	+	−	−

TABLE VII

Precipitin Reaction of the Digestate of Preps. III's from Group A Gastric Cancer and Group A Mucosa together with that of the Undigested Materials with the Anti-cancer and Anti-mucosa Sera in Table V

The mixture incubated for digestion, 30 mg. Prep. III +35 mg. trypsin in 1.5 cc. water; duration of incubation, 30 days. −: no precipitin reaction, +: occurrence of the reaction.

Antigen from		Dilution of antigens (1 :)						
		50	10^2	2×10^2	4×10^2	5×10^2	6×10^2	10^3
Anti-cancer serum								
Group A cancer	digested	−	−	−	−	−	−	−
	undigest.	+	±	−	−	−	−	−
Group A mucosa	digested	−	−	−	−	−	−	−
	undigest.	±	±	−	−	−	−	−
Anti-mucosa serum								
Group A cancer	digested	−	−	−	−	−	−	−
	undigest.	−	−	−	−	−	−	−
Group A mucosa	digested	−	−	−	−	−	−	−
	undigest.	±	−	−	−	−	−	−

gastric cancers and Group A gastric mucosa and Preps. II's and III's from Group A gastric cancers and Group A gastric mucosa were subjected to digestion. Tables V–VII illustrate the results by the digestates together with those by the undigested materials.

The digestate of Preps. I's and II's showed generally as strong precipitin reaction as the undigested, except when the digestate of Prep. II of the cancers (Group A) was laid over the anti-mucosa serum, which is difficult to explain without a further study. Regarding Preps. III's, the sera were too weakly reactive with them as such to draw any conclusion from the negative reaction of these specimens after digestion.

3) For the skin test a part of the digestates above were diluted to 0.1–0.05% with physiological saline and a 5% phenol solution as above, and the technique of the assay above was also followed. The results are diagrammatically expressed in Figs. 4–6. It was revealed that the digestion had not destroyed the structure giving the cancer reaction at all.

SUMMARY

1. In iso- and homologous hemoagglutination-inhibition, the protein

Fig. 4

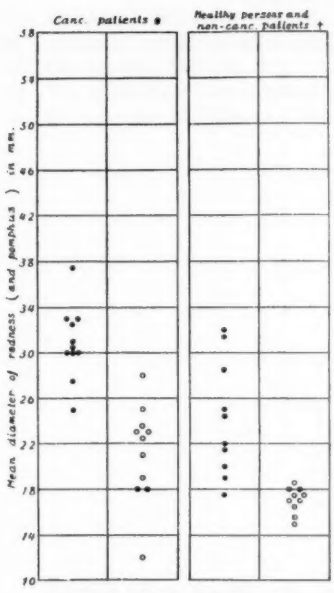

Fig. 5

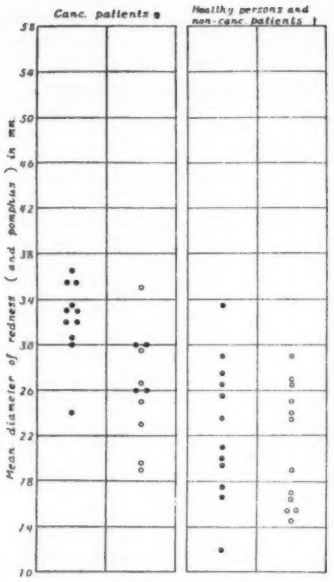

Fig. 4. Showing the skin reaction with the digestate of the materials which gave the results in Fig. 1.

The digestates were diluted for the test as much as the undigested.

• the digestate of the cancer substance. ○ the digestate of the mucosa substance. * carcinoma of stomach (2), maxillary antrum (2), oesophagus (1), gall bladder (1), biliary ducts (1), liver (1), pancreas (1) and mamma (1), and sarcoma (1). † healthy persons (2), hernia (1), gastric ulcer (2) and duodenal ulcer (5).

Judgement: the digestate of the cancer substance, positive; the digestate of the mucosa substance, positive.

Fig. 5. Showing the skin reaction with the digestate of the materials which gave the results in Fig. 2. The digestates were diluted for the test to 0.05%.

• the digestate of the cancer substance. ○ the digestate of the mucosa substance. * carcinoma of stomach (2), maxillary antrum (2), oesophagus (1), gall bladder (1), biliary ducts (1), liver (1), pancreas (1) and mamma (1), and sarcoma (1). † healthy persons (5), duodenal ulcer (4), gastric ulcer (2) and hernia (1).

Judgement: the digestate of the cancer substance, positive; the digestate of the mucosa substance, positive (not so distinct).

fractions of A and O gastric cancers were less potent than the corresponding protein preparations from gastric mucosa of respectively same blood groups.

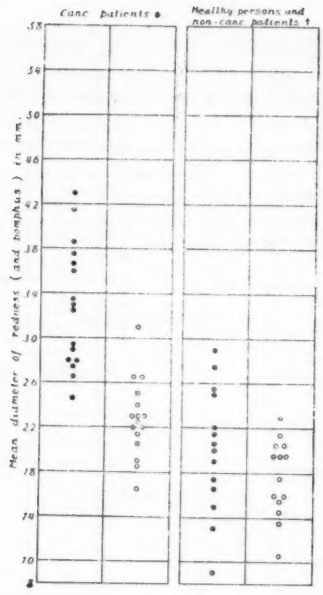

Fig. 6. Showing the skin reaction with the digestate of the materials which gave the results in Fig. 3 (both diluted to 0.1%).

• the digestate of the cancer substance. ◦ the digestate of the mucosa substance. * carcinoma of mamma (4), rectum (2), uterus (2), stomach (1), maxillary antrum (1) and tongue (1), and sarcoma (5). † healthy persons (9), gastric ulcer (1), haemorrhoids (1), anal fistula (1), cholelithiasis (1) and vesical papilloma.

Judgement: the digestate of the cancer substance, positive; the digestate of the mucosa substance, positive.

2. As to the precipitin reaction with anti-cancer and anti-gastric mucosa rabbit sera, the preparations indicated that immunological divergency existed in at least some pairs of the corresponding proteins from the two sources.

3. In respect with the hemolytic power, the preparations from the both kinds of tissue appeared to be nearly devoid of it.

4. The cancer substances proved more powerful in giving cancer skin reaction than the corresponding mucosa substances, although both groups of the preparations had this potency, of which they were not deprived by trypsin.

5. Taken altogether, the stomach cancer proteins are not regarded identical with the corresponding proteins of stomach mucosa from the biological view point too (Cf. the foregoing report).

References

1) Masukawa, Tohoku J. Exp. Med., this volume, p. 251.
2) Masukawa, *ibid.*, this volume, p. 261.
3) Oh-Uti, *ibid.*, 1949, **51**, 302.
4) The immune sera were obtained by injection of the physiological saline tissue extracts.
5) Cf. Kobayasi, K., Tohoku J. Exp. Med., in press.

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Microscopical Study of Oculomotor Nucleus in Human Adult

I Report

By

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Many histological studies on the oculomotor nucleus in man have been reported of old, but most of them were carried out with general staining methods, scarcely any one of them being done with special staining methods for neuro-fibrils. Then, I took my materials from 3 adult midbrains, fixed them for a long time in 10% neutral formol, and upon slicing one of them into transversal and two of them into sagittal 40 μ frozen sections, stained all of them with Seto's silver impregnation. These preparations I subjected to minute microscopic study and arrived at the following results.

Individual Views

The oculomotor nucleus in human adult is on the level of colliculus rostralis. The upper end of the nucleus approaches the bottom of 3rd ventriculus in the vicinity of commissura caudalis and the lower end the top of colliculus caudalis, lying adjacent to the inner ventral edge of stratum griseum centrale surrounding aquaeductus mesencephali. The length of the nucleus in sagittal section has been measured variously by Kölliker[1] and Krause[2] at 5–6 mm. and Obersteiner[3] at 6–8 mm., but my measurement resulted in nearly 6 mm. length for all the specimens. The area of the nucleus in transversal section, according to Krause, was about 3 mm^2., but I found it larger, i.e., about 5 mm^2. The oculomotor root fibres, according to Krause, numbered approximately 15,000, which statement I could not verify to satisfaction.

As widely known, the division of the nerve cell groups composing the oculomotor nucleus has been undertaken by Westphal[4], Perlia[5], Edinger[6], Mingazzini[7] and others. The inner or the inner dorsal portion consists of a pair of small cell groups, called parasympathetic nuclei, lying in the stratum griseum centrale on both sides of the median line,

which Jacobsohn[8] named sympathetic nuclei and Obersteiner[3], the small cellular median nuclei. The outer or the outer ventral portion consists of a pair of large cell groups, generally called the chief nuclei, but the nuclei laterales by Obersteiner[3], Edinger[6] and Tsuchida[9] and the lateral chief nuclei by Kölliker[1]. Besides, there is seen the so-called median nucleus between the chief nuclei of both sides and on the ventral side of the nuclei parasympathici, extending across the median line. This nucleus is not in pair and was called the nucleus centralis by Perlia[5], and the nucleus medianus impar by Bernheimer[10] (Fig. 1).

A. Chief nucleus or Lateral nucleus

The shape of the chief nucleus in transversal section is described as comma-shaped by Monakow[11] and crescent-shaped by Mingazzini[7], but my observation showed its lower part to be triangular with the apex directed ventralward and circumscribing the fasciculus longitudinalis medialis, and its upper part to form an elongated ellipse, independent of the said fasciculus, since the latter gradually parts from the nucleus at higher levels.

This nucleus was thought to be homogenous by Cassirer and Shiff[12], Bernheimer[10], and Siemerling and Boedeck[13], but Edinger[6], Obersteiner[3] and Bach[14] divided it into the ventral and dorsal parts and Mingazzini[7] and Kölliker[1] classified it into four cell groups, dividing the two parts again into the upper and the lower. My observations on the subject is as follows. The classification of the cell groups can be established by the slide series of transversal and sagittal section, and it seems most appropriate to divide the nucleus into the three parts, that is, the ventral, dorsal and caudal parts.

In the rostral levels of the chief nucleus, the dorsal part is on the dorso-lateral side of the nucleus, and shorter and smaller than the ventral part, shaped almost spherical, and better provided with nerve fibre nets than the latter. The dorsal part is demarcated by the surrounding coronal fibres so distinctly, that the division from the ventral part is very clear. The ventral part consists of more numerous but more sporadic nerve cells than the dorsal part, the nerve fibre nets here being more scattered and less curved in their course.

On the contrary, in the lower levels of the chief nucleus, the division between the ventral part and the dorsal part disappears, and the nerve cell group in sagittal section, as shown in Fig. 2, displays a specialized conformation apparently being represented by a separate group as distinguished from the ventral and dorsal parts. From such observations I was led to propose a new division of a caudal part apart from the ventral and dorsal parts.

Now, the nerve cells in the chief nucleus are mostly stellate, but

Fig. 1

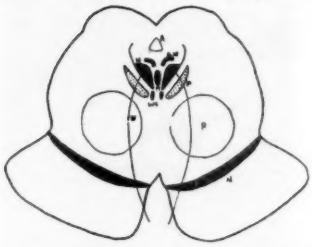

Fig. 2

Fig. 1. Schema of horizontal section of mesencephalon in man at the level of colliculus superior. A aquaed. mesenceph.; Ew nucl. parasymp.; H chief nucleus; M nucl. medianus; VM nucl. anteromedialis; F fasc. longit. med.; W oculomot. nerve root; R nucl. ruber; N nucl. niger.

Fig. 2. Sagittal sections of the chief nucleus of oculomotor mucleus in human adult. The nucleus appears in its 3 salient parts. V ventral, D dorsal and C caudal part.

sometimes pear- and club-shaped, and provided with many stout nerve processes, showing as a whole a form of rugged virile motor nerve cells, which resemble the motor cells in the nucleus n. hypoglossi in man (Sato[15]) very much. They are easily distinguishable from the round feminine multipolar sensory nerve cells found in the brain trunk.

The size of these motor cells is according to Obersteiner[3] 50 μ, and by my examination 60 μ in the maximum and 40 μ in the minimum. The nerve fibrils are arranged reticularly in the cell bodies, but in the processes they are spread out at length, without changing in nature. To distinguish the long processes from the short processes is often difficult, but in general the former have lightstaining basal cone in their beginning, which passes into very thin fibre. But this fine fibre becomes strong and more deeply stainable, as soon as it is provided with the myelin.

The long process is one per cell in general, but since sometimes two or bifurcated long processes are observed emerging from one cell, the total number of the oculomotor root fibres is accordingly larger than the total number of the nerve cells. The short processes exist in any number from 1 to 6 per cell and they are thicker than the long processes, but stain only weakly. In most cases, they branch out again either after a prolonged course upon emerging from the mother cells, or after a short course. The terminal fibres of the short processes end sometimes unbranched, but sometimes furthermore branched out into minute rami. But I regret to say I could make no further observations on the terminal formation. The cell nucleus of the motor nerve cells is relatively small and located near the center of the cell, and contains a number of deeply stainable granules and one evident nucleolus (Figs. 3 and 4).

Fig. 3

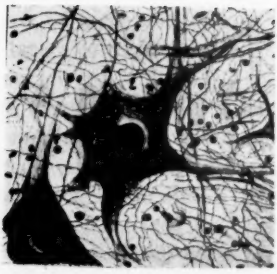

Fig. 4

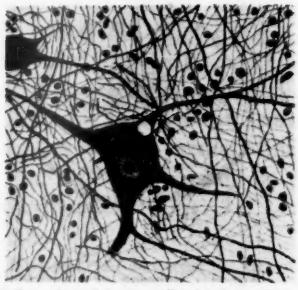

Fig. 3. Motor cells in oculomotor chief nucleus in man. Sagittal section. Very complex intranuclear plexus is seen spread out in glia tissue surrounding the nerve cells and their numerous processes. Thick fibres represent motor and sensory fibres, while fine fibres vegetative fibres. Seto's silver impregnation. X 500, reduced to 2/3.

Fig. 4. Ditto. The long process running to the upper right is seen divided into 2 branches before running long. Same staiing. X 300, reduced to $\frac{1}{2}$.

In the chief nucleus Tsuchida[9] has found the smaller cells between the large motor nerve cells, similar to those found by Siemerling and Boedeck[3], and Bach[14] in the abducens nucleus, and had his doubt of the motor nature of them. Suzuki[16] has assumed that these nerve cells represent the centre for the ocular tension and are of extrapyramidal nature, upon experimental research on dogs.

I could ascertain the existence of smaller cells in the chief nucleus, as shown in Fig. 5, which are of size $\frac{1}{2}$–1/3 of the motor cells, pear-shaped, somewhat smaller but rounder than the parasympathetic cells of nucleus parasympathicus described hereunder, and are either unipolar or bipolar, with globular nucleus located at or near the center of the cells. The cell nucleus is rather large and the protoplasma is consequently small in quantity, a peculiarity of these cells. From such observations, I conclude that these smaller cells are not motor in nature but are vegetative cells morphologically.

I could also find some nerve cells (Fig. 6), seemingly of sensory nature, at the caudal part of the chief nucleus. They are nearly of the same size as the motor cells, are globular, mainly bipolar and strongly stainable, with one each cell nucleus somewhat smaller than that in motor cells at the center and containing one nucleolus. No description of such cells in the chief nucleus has been known, but the above observations show them to be similar to the sensory nerve cells I found in the nucleus mesencephali n. trigemini[17], and I assume they must be similarly of sensory nature. Beattie[18] found some centripetal nerve fibres seemingly terminating in nucleus mesencephali n. trigemini in the oculomotor root fascicle,

Fig. 5

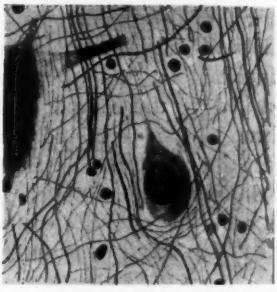

Fig. 6

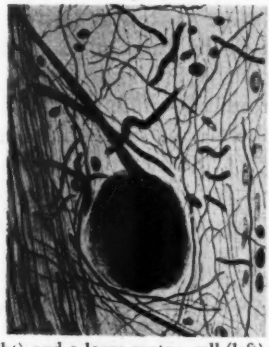

Fig. 5. A small vegetative nerve cell (right) and a large motor cell (left) in the oculomotor chief nucleus. Sagittal section. Same staining. X 500, reduced to 2/3.

Fig. 6. A sensory nerve cell seen in the oculomotor chief nucleus, same section. F fasc. long. med. consisting of thick and thin fibres. Same staining. X 500, reduced to 2/3.

and suggested that they have proprioceptive function. But I believe at least some of these fibres stand in deep relation with the sensory nerve cells I discovered.

In the inner and the ventral parts of fasciculus longitudinalis medialis, the so-called lateral cells by Bernheimer[10], the " Bucht " cells by Monakow[11], or the accessory cells by Kölliker[1], are said to exist. Krause[2] failed to clarify their nature, but Kölliker[1], Bernheimer[10] and Bach[14] agreed in assuming their motor nature, similar to the nerve cells of the oculomotor nucleus. I also succeeded in observing the existence of not only motor nerve cells similar histologically to those in the chief nucleus at all its levels at the same places, but also of smaller cells of vegetative nature. These nerve cells are spread along the oculomotor root fascicle running through the fasciculus longitudinalis medialis, sometimes forming small groups.

Whether the chief nucleus is divisible into the respective motor centres for the external ocular muscles, has been discussed in the numerous reports by Koehler and Pick[19], Darkschwitsch[20], Siemerling[13], Bach[14], Panegrossi[21], Cassirer-Schiff[12], Majano[22], Bernheimer[10], Monakow[11], Tsuchida[9] and Breuwer[23], that of the experimental study on monkeys by Bernheimer in recent days deserving special mention. According to Bernheimer the chief nucleus is areally divided into motor centres, from top downwards, for m. levator palpebrae superioris, m. rectus bulbi superior, m. rectus bulbi nasalis, m. obliquus bulbi inferior and m. rectus bulbi inferior in the order named, the upper two centres innervating

only the muscles on the same side, the next two innervating the muscles on the same as well as on the opposite side, the last one controling only the muscle on the opposite side, in exclusion of the same side.

However, Siemerling[13] and Mingazzini[7] refused the proposed mapping as anatomically unfounded. I also am inclined to look upon the map of Bernheimer as somewhat schematic, considering the complicated intertwining of the nerve fibres in the chief nucleus, ascertained upon the minute scrutiny of the series of my preparations. I would rather have it that the nerve cells innervating the various muscles, instead of being confined to limited special areas, are rather widely spread out, mutually encroaching upon the adjacent central cell groups, and overlapping each other in comparatively wide areas.

B. Nucleus medianus

The median nucleus is in the horizontal section club-shaped according to Obersteiner[3], cigar-formed according to Monakow[11] and almond-shaped according to Mingazzini[7], but I would rather describe it as spindle-form. It lies on the median line between the chief nuclei of both sides, and its lateral sides are surrounded by the nerve fibres running parallel in a ventro-dorsal direction. According to Obersteiner, it is not clearly distinguishable from the chief nuclei. Tsuchida[9] also says that in 20% of human specimens, the boundary of this nucleus is indistinct. My observations showed that the boundary between the median nucleus and the chief nucleus becomes more accentuated as the upper level is reached, while at the power part of these nuclei, their nerve cells mutually pass over from the one to the other, somewhat blurring out the boundary.

Perlia[5] and Bernheimer[10] assert that the nerve cells in the nucleus medianus are histologically similar to the motor cells in the chief nucleus, but in my observations, I found that the former are in the upper part of the median nucleus somewhat different from the latter, though they show in the caudal part more or less similarity to the motor cells in the chief nucleus. Namely, in the median nucleus, multipolar cells diminish gradually as the upper levels are reached, being taken place by many bipolar club- and spindle-shaped cells. The processes of the multipolar cells found here are smaller in number and shorter in length, their protoplasm less stainable than in the cells of the chief nucleus, but the cell nucleus is somewhat bigger (Figs. 7 and 8). The nerve cells in this part are not scattered in arrangement as in the chief nucleus, but are arranged in parallel lines with their long axis oriented ventro-dorsally. Beside the two kinds of cells described above, I found the existence of nerve cells very similar to the vegetative small cells found in the chief nucleus. They are either unipolar or bipolar, a little smaller than the latter, but with somewhat larger cell nucleus. Thus the nucleus medianus contains cells

Fig. 7

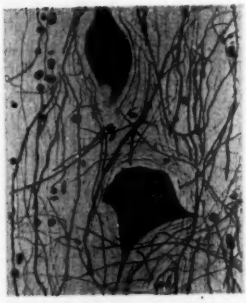

Fig. 8.

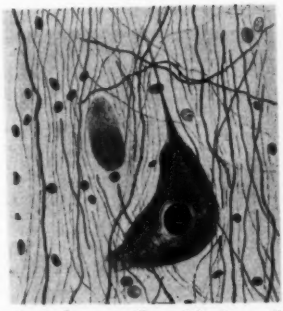

Fig. 7. A specific bipolar cell (upper) and a small multipolar cell (lower) in the upper part of the oculomotor median nucleus. Horizontal section of human adult mesencephalon. Same staining. X 500, reduced to $\frac{1}{2}$.

Fig. 8. A small vegetative nerve cell and a motor nerve cell in the oculomotor chief nucleus. Same section. Same staining. X 500, reduced to 2/3.

of three types.

Bernheimer[10] supposed that this nucleus, together with the parasympathetic nucleus, are connected with the inner ocular muscles, a supposition thrown into doubt by Majano[21], Marina[24], Edinger[6], Brouwer[23], Monakow[11] and others, who maintained that this nucleus controls the convergence action in connection with the outer ocular muscles. But some neurologists, including Frank[25], deem it probable that the convergence control lies with the parasympathetic nuclei. Anyhow, in consideration of the complex cellular structure of this nucleus, it may be surmised that the function of this nucleus is far from a simple nature.

C. Nucleus parasympathicus (Edinger-Westphal's nucleus)

This pair of nuclei, discovered by Edinger in human embryo and by Westphal in human adult, is located near the median line at the level of the transitional part from ventriculus tertius to aquaeductus mesencephali, more closely conjugated than the chief nuclei of both sides. This nucleus is situated at the upper dorsal side of the chief nucleus, curved in an arc in transversal section, showing a specific histological picture of rough lightness, clearly distinguishable from the surroundings.

The nerve cells are generally small as shown in Fig. 9, being about 1/3 in size of the large cells in the chief nucleus. They are either unipolar or bipolar, narrow and long, sometimes elliptical, spindle-form or pear-shaped, one of the poles being round and the other generally more pointed. These oblong cells are arranged rather densely in the direction conforming

Fig. 9

Fig 10

Fig. 9. Nerve cells in the oculomotor parasympathetic nucleus in horizontal section of adult human mesencephalon. Note the big size of the cell nucleus. Same staining. X 800, reduced to 2/3.

Fig. 10. Oculomotor root fibres in the same section. Thick fibres show motor fibres and thin ones vegetative fibres. Same staining. X 500, reduced to $\frac{1}{2}$.

with the bow-shape of the nucleus, giving of the appearance of a file of fish in shoal.

One of the outstanding characteristics of these nerve cells is the largeness of the cell nucleus. It is generally placed excentrically in the cells, is globular or oval in shape, and the boundary between the surrounding weakly stainable protoplasm is very distinct. The nucleolus is globular, located near the center of the nucleus and strongly stainable.

Since Edinger[6] and Westphal[4] concluded that this nucleus represents a centre for the pupil, Koehler and Pick[19], Darkschwitch[20], Bernheimer[10] and Levinsohn[26] proposed that it is a centre for the contraction of pupil, but Juliusburger and Kaplav[27], Cassirer and Schiff[12], Panegrossi[21], Majano[22], Bach[14], Monakow[11] and Tsuchida[9] refuse to accept the proposal. Recently Ogawa[28] seems also to doubt the validity of the above proposal, from a view point of comparative anatomy. In my observation, as shown in Fig. 10, I found many minute fibres commingling in the oculomotor root, and I have no doubt that the long processes from this nucleus pass into such minute fibres, representing one of the important elements of the oculomotor nerve.

I discovered the existence of a new small cell group (Fig. 11) on the upper dorsal side between the fasciculus longitudinalis medialis and the chief nucleus. These cells histologically agree with the cells in the parasympathetic nucleus, are arranged in group or sometimes sporadically, in a number around 20 in transversal section. I wish to call this nucleus the accessory nucleus of nucleus parasympathicus.

Fig. 11

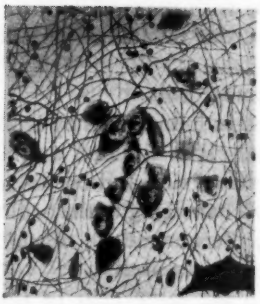

Fig. 12

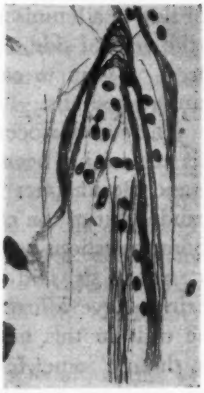

Fig. 11. Group of small nerve cells in my so-called nucleus accessorius of oculomotor parasympathetic nucleus in the same section. Multipolar motor nerve cells are seen in the lower right corner. Same staining. X 300, reduced to $\frac{1}{2}$.

Fig. 12. Same nerve cell group and fasc. lemniscularis in the oculomotor antero-medial nucleus in the same section. Same staining. X 160, reduced to 1/3.

D. Nucleus anteromedialis

This nucleus (ventral cell group by Mingazzini[7]) is located on the ventral side between the fasciculi longitudinales mediales of both sides at the level of the upper part of nucleus medianus, is elliptical in transversal section, with the long axis running ventro-dorsally, and bounded on the outer side by a multitude of fine nerve fibres. This nucleus has been excluded from the oculomotor nucleus by Cassirer-Schiff[12], Siemerling-Boedecker[13] and Monakow[11], but assumed to be the proximate column of nucleus parasympathicus by Edinger[13], Obersteiner[3] and Greving[29]. According to Perlia[5], Tsuchida[9], Panegrossi[21], Frank[25] and Mingazzini[7], it belongs to the oculomotor nucleus, but is independent of the parasympathetic nucleus.

In my study, I observed that this nucleus is analogous to the nucleus parasympathicus in the form and arrangement of the cells. Mingazzini[7] failed to elucidate the connection of this nucleus and the parasympathetic nucleus, but I found evidence of the union through a cell-bridge containing these parasympathetic cells in sagittal section. From this fact it may be deduced that these both nuclei are of the same nature. According to a recent study by Frank[25] this nucleus also has a close relation with the convergence and regulative action of the ocular muscles.

I also succeeded in discovering medullated thick fibres surrounding the antero-medial nucleus (Fig. 12). These fibres originate in the outer

ventral side of the nucleus, run to the dorsal side thereof to circumscribe its dorsal side in a lemniscus, then run through the inner side of the nucleus toward the ventral side, apparently to mingle with the oculomotor medial root fibres. I wish to call this fibre bundle the fasciculus lemniscularis nuclei anteromedialis.

E. Nucleus dorsocentralis (Nucleus Panegrossi)

This nucleus has been found by Panegrossi in human and by Kölliker[1], Bach[14] and Bernheimer[10] in animal brains. It is situated at the level of the caudal pat of the chief nucleus on its dorsal side and on the median line. Some questions have been cast upon this belonging to the oculomotor nucleus, but it is said that Panegrossi[21], Bernheimer[10], Bach[14] and Mingazzini[7] have affirmed it by experimental research.

The cells in this nucleus are similar to the large cells in the chief nucleus, though somewhat smaller in size. They are arranged rather in scattered positions, so that the boundary of this nucleus lacks definition. In this nucleus, some smaller vegetative cells, similar to those in the cheif and median nuclei, have been observed, though in a small number. From such observations, it could be concluded that this nucleus is also a motor centre for the outer ocular muscles, like the chief and median nuclei.

Summary

The oculomotor nucleus comprise the chief nucleus, the median nucleus, the parasympathetic nucleus, the anteromedial nucleus, the dorsocentral nucleus and the accessory nucleus of the parasympathetic nucleus, the last of my own discovery.

The nerve cells in the chief nucleus are represented in their majority by large motor cells provided with many processes and showing virile appearance. The long processes being sometimes double and some-times bifurcated per cell, the total number of the oculomotor root fibres is larger than that of the cells. The short processes apparently end sharply, either unbranched or branched, but their terminal formation is not enough clarified. The cell nucleus is comparatively small and located near the centre of the cells.

The cells of the chief nucleus are partially represented by smaller cells, $\frac{1}{2}$ to 1/3 in size of the large cells, pear-shaped in form, unipolar or bipolar, with a large cell nucleus, presumably being of vegetative nature. Cells of a third type are extremely few in number. Their size is not much different from the large motor cells, but in form, they are globular and unipolar. They are very similar to the sensory nerve cells found in the nucleus mesencphali n. trigemini, and are without doubt of sensory nature.

In the inner and the ventral parts of the fasciculus longitudinalis medialis are found some cells of the same form as the motor cells in the

chief nucleus, with which a small number of small vegetative cells are commingled.

It seems impossible to divide the chief nucleus distinctly into the various motor centres having each fixed boundary for the outer ocular muscles, because they may be thought diffused over comparatively wide parts, overlapping each other to an extent.

The nucleus medianus is inseparable from the chief nucleus at the caudal pats but the boundary between them become more definite as the higher levels are reached. The nerve cells in the basal parts are similar to those in the chief nucleus, but in the upper reaches, more and more bipolar club- or spindle-like cells come into evidence. Also, some smaller cells resembling vegetative nerve cells in the chief nucleus are observable.

The cells in the parasympathetic nucleus are small, about 1/3 of the size of the motor cells in the chief nucleus, are unipolar or bipolar, oblong in shape and arranged rather densely in the direction conforming with the arcuate form of this nucleus. Their protoplasm is only weakly stained, their cell nucleus is oversized, and their long processes are represented by minute fibres. Since such minute fibres are also observable in the oculomotor nerve root, I am inclined to give adherence to the proposal that this nucleus is the centre for the pupillar contraction.

I have discovered a small group of nerve cells strongly resembling those in the parasympathetic nucleus on the rostrodorsal side between the fasciculus longitudinalis medialis and the chief nucleus. I propose to call this new nucleus the accessory nucleus of the parasympathetic nucleus.

The anteromedial nucleus is of the same nature as the nucleus parasympathicus, considering the form and the arrangement of the cells. This fact is further evidenced by the union of this nucleus with the parasympathetic nucleus through a cell-bridge containing such cells.

The nerve cells in the nucleus dorsocentralis are somewhat smaller than, but similar to, the motor cells in the chief nucleus. In this nucleus, some smaller vegetative cells, as have been found in the chief and the median nucleus, are observable in a small number. Thus, this nucleus also may be assumed to represent one of the oculomotor centres.

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Microscopic Study of Oculomotor Nucleus in Human Adult

II Report

By

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(Received for publication, February 3, 1953)

I have reported in the previous number of this series of reports on the minute structure of the nerve cells in the various parts of oculomotor nucleus in human adult[1]. In this report, I will detail the external nerve fibres, the intranuclear nerve plexus and the oculomotor nerve root conerned with the oculomotor nucleus.

A. External nerves

Entrance of motor nerve fibres from the tractus corticobulbaris of tractus pyramidalis as well as sensory nerve fibres connected with the ocular reflex into the oculomotor nucleus must be expected, as in all other cerebrospinal motor nuclei, but the routes of such incoming nerve fibres have remained very obscure to date. In particular, the course of the motor nerve fibres have never been exhaustively inquired into.

According to my observations, the motor nerve fibres seem to be represented by a number of nerve fibres originating in the tractus pyramidalis cruris cerebri, running along the rhaphe with or without crossing and reaching the chief and median nuclei of oculomotor nucleus, while the sensory nerve fibres are represented by the partially intercrossing thick fibres originating in the stratum griseum centrale, and the strongly staining thick fibres running longitudinally through the chief nucleus, but I feel some incertitude on the accuracy of the above description.

Thus I failed to make a satisfactorily clear observation on the ending formation in the oculomotor nucleus of the incoming motor and sensory fibres, but I could neither observe any of the extremely simple and rough reticular terminations around the cells, nor the neural connection of the external nerve fibres and the nerve cells. On the contrary, the incoming nerve fibres appear to branch or anastomose in the neuroglia tissue and make the transition into the intranuclear plexus described hereunder. Consequently, the transmission of impulses to the oculomotor nucleus,

similar to the cortex cerebelli (Yamamoto[2]) and to the nucleus nervi hypoglossi (Sato[3]) seems to be effected by the stimuli coming over the external nerve fibres exciting the neuroglia tissue which is probably endocrine in nature, the endocrinal secretion serving to excite the nerve cells and their short processes secondarily, the stimulation being then transmitted peripherally through the long processes.

B. Intranuclear nerve plexus

The nerve cells in the oculomotor nucleus, together with their processes, are buried in a system of complex nerve plexus, but the nature of this plexus has never been histologically elucidated. According to what I have observed in my study, this plexus is a very complex formation composed of incoming motor, sensory and vegetative nerve fibres around the various nerve cells and their processes in the oculomotor nucleus. However, I failed in exposing the details of the terminal formation of the various kinds of nerve fibres. For example, it was sometimes impossible to decide whether the terminal branches of these fibres end freely or in reticular formation. But it is beyond doubt that the figures of strongly staining thick fibres and their sections represent motor and sensory nerve fibres, and the minute fibres arranged reticularly derive from incoming vegetative nerve fibres.

C. Oculomotor nerve root, especially, decussating fibres (Figs. 1 and 2)

Fig. 1

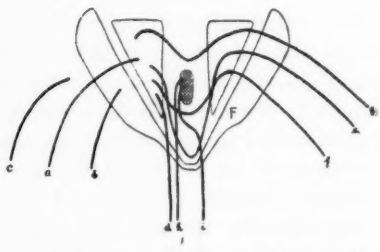

Fig. 2

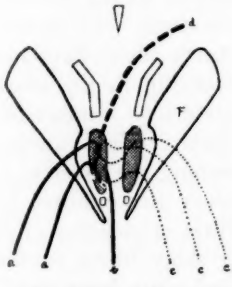

Fig. 1. Schema of course of motor fibres from the motor nuclei of oculomotor nucleus in man. Horizontal section at the level of the lower part. a, b, c lateral non-decussating fibres, d median non-decussating fibres, and e, f, g decussating fibres from the chief nucleus; h non-decussating fibres and i decussating fibres from the median nucleus; F fasciculus longitudinalis medialis.

Fig. 2. Schema of course of motor nerve fibres from the chief nucleus of oculomotor nucleus in the same section as above. a lateral non-decussating fibres, b median non-decussating fibres and c decussating fibres; d sensory nerve fibres.

In horizontal sections, the oculomotor root bundle shows in general a characteristic S-formed curve outwardly, after having penetrated the fasciculus longitudinalis medialis, but at the lowermost part, no formation of root bundle is seen. The root fibres run along the base of the nucleus ruber, then through the centre of the nucleus niger. At higher levels, the fibres run along the inner side wall of the nucleus ruber and emerge through the substantia alba near the median line. The root fibres in the topmost part penetrate the substantia alba further adjacent to the rhaphe.

On the decussating fibres in the root bundle opinions are yet divided. First, Gudden[4] observed partial decussation in vertebrates, and Perlia[5], Kölliker[6] and van Gehuchten[7] in man. Kölliker assumed their origin in the outer ventral cell group of the chief nucleus and Perlia and van Gehuchten in the dorsal cell group of it. Obersteiner[8] asserted that all decussating fibres originated on the dorsal side only, but Bernheimer[9] suggested that the lower part of the oculomotor nucleus sends out decussating fibres only, the middle part, decussating as well as non-decussating fibres and the upper part, mainly non-decussating fibres, so decussating fibres come from the dorsal as well as the ventro-caudal sides of the nucleus.

My study showed that decussating fibres greatly predominate in the lower part of oculomotor nucleus, only few non-decussating fibres being found. The decussating fibres originate in circa 2/3 of the dorsal area of the chief nucleus, but a part also comes from 1/3 of its ventral area. The course of these decussating fibres is, first toward the inner ventral part of the same side, then abruptly to the lower caudal part down to the median line a little above the caudal end of the fasciculus longitudinalis medialis and thence, turning in an acute angle, running in a lemniscus up to the opposite side, to penetrate through the chief nucleus on that side and finally enter the fasciculus longitudinalis medialis. In the upper regions, the decussating fibres become extremely small in number, but emerge equally from all sides of the chief nucleus, and attain the opposite side without running any lemniscular course as described above. Also some of the fibres derived from the nucleus medianus seem to decussate.

Summary

In probability, the motor nerve fibres coming from the tractus pyramidalis cruris cerebri are represented by the nerve fibres running along the rhaphe to the chief and median nuclei after or without decussating, while the sensory nerve fibres are represented by the fibre group coming from the stratum griseum centrale partially decussating and fibres running longitudinally through the chief nucleus, but many points are not yet in the clear on the subject.

The ending formation of the external motor and sensory nerve fibres in the oculomotor nucleus could not be elucidated satisfactorily, but I certainly could not observe any simple terminal networks around the cells and others as would have been expected according to the Langley's neuron theory. On the contrary, these fibres, after branching and anastomosis in the neuroglia tissue, seem to go over into extremely complex intranuclear plexus found around the nerve cells and their processes. Though many points remain to be clarified regarding the formation of this plexus, it is at least certain that the strongly stainable thick fibres represent motor and sensory nerve elements and the minute fibres showing fine reticular formation, vegetative nerve elements.

The majority of the oculomotor nerve root is composed of decussating fibres at the lower part of the oculomotor nucleus, originating mainly from the dorsal side of the chief nucleus and partly from the ventral side thereof. These head first toward the inner ventral part of the same side, then abruptly drop to a point a little above the lower end of the fasciculus longitudinalis medialis, cross over the median line, return upwards to penetrate the chief nucleus of the opposite side and enter the fasciculus longitudinalis medialis. In the upper part of the nucleus the decussating fibres are found extremely small in number. They emerge from various sides of the chief nucleus and attain the opposite side without running such a devious course. Also some of the median fibres coming from the upper part of the median nucleus apparently decussate in their course.

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A Modified Stereotaxic Apparatus* for Brain Surgery of Deep Portions

By

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(Received for publication, February 5, 1953)

About forty years have passed since the first report of the so-called Horseley-Clarke stereotaxic apparatus.[1] In 1947 its modified apparatus for the human brain was for the first time designed by Spiegel *et al.*[2] Thereafter several kinds of such a stereoencephalotome have been described by Hyne *et al.*[3] in the United States, David *et al.*[4] in France, and Uchimura and Narabayashi[5] in our country.

We have already experienced the dorsomedial thalamotomy,[6] an operation to put a certain surgical invasion upon the deep part, particularly the dorsomedial part of the thalamus. However, it has, step by step, become necessary to perform this operation by using such an apparatus that makes it possible to perform an accurate measurement of any portions within the human brain. For such a purpose, we have made many trials for designing its apparatus and at last we have been able to produce the one with several modified points. Here we wish to exhibit it.

The designed apparatus is shown in Figs. 1 and 2. This apparatus has, we have thought, several improved points, when compared with those already reported by the other authors. The main points having differences are as follows:

1) The most conspicuous point would be that serious efforts have been made to produce it as strong and solid as possible: for this purpose, many peculiar considerations were paid from standpoint of the dynamics. It is forty cm. cube and is 12 kg. in weight. 2) The construction is so simple that man can treat it easily and briefly. There are many screws at the points of connection and man can adjust any parts of the apparatus according to requests. 3) Before the beginning of the operation, the basic part of the apparatus is immediately connected to the operation table, and then many surgical manipulations take place. And, finally,

* This apparatus has been produced under many technical assistances by Dr. K. Shinotsuka of the Institute for Scientific Measurement, Tohoku University.

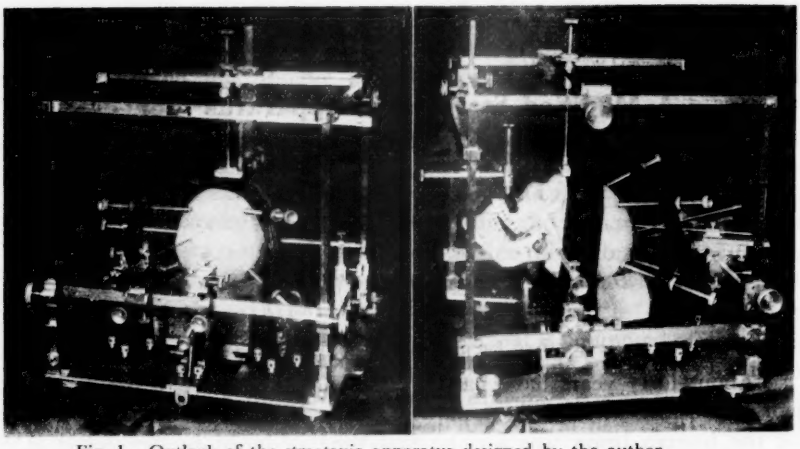

Fig. 1. Outlook of the streotaxic apparatus designed by the author.

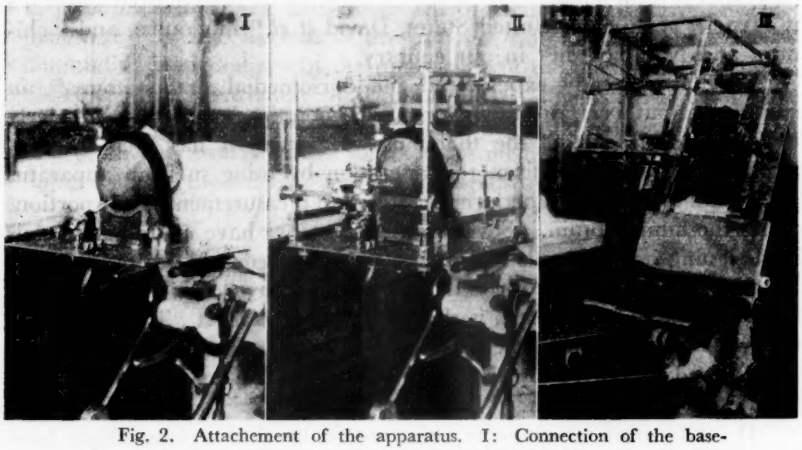

Fig. 2. Attachement of the apparatus. I: Connection of the base-plate to the operation table and fundamental fixing of the patient's scalp; II: Complete connection and fixing; III: Attachement in sitting position.

the upper construction necessary for measurement is put on. Therefore, a fixing of the apparatus is not only stable but also easily manageable. Furthermore, it would be characteristic that none of the load of the weight of the apparatus id added to the patient on the operation table. 4) A pair of attachements for ear-holes and that for grabella are necessary for the measurement and determination of basic points, however they are applicable as fixing equipment for the scalp, too. 5) We added a device for adjusting the face, besides the scalp: viz. a fixing equipment for maxilla

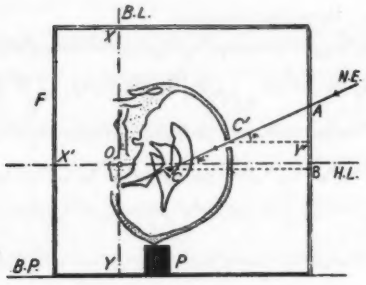

Fig. 3. Scheme showing the direction of inserting electrode (E.N.). B. P.: Base-plate to be connected to the operation table; F: Flame; O: Ear-hole; A: Trephined hole; C: Aimed point, viz. dorsomedial part of the thalamus; P: Wooden pillow; $\overline{XY}$: The base-line (B.L.); $\overline{X'Y'}$: Horizontal line (H.L.). $\overline{BC}$ is parallel to $\overline{X'Y'}$ and $\angle XOY'$ is rectangular. However, the point C′ is a fixed point. Accordingly, ΔABC would become to be fixed, if $\angle ACB$ is determined. Here, $\angle ACB$ is able to measure by x-ray. Therefore, in case of operation, only $\angle ACB$ is measured and adjusted by means of the apparatus.

(s. Fig. 1). This seems very useful in case of operative manipulation. 6) We employed a pair of electrode-holders which are movable along a micrometer and also movable to any desired direction, viz. universal. Also, this holder is removable and, therefore, an insertion of electrodes from behind, both sides and upper spheres to the scalp, can be performed. 7) By moving the operation table, an attachement of the apparatus, or an operation at any patient's positions, becomes possible of taking place according to the operator's request: an operation at sitting position is, of course, possible (s. Fig. 2).

Using such an apparatus as has been above described and employing electrodes for producing lesions, we are now performing operations in the deep portions of the brain. In case of the dorsomedial thalamotomy operation, we attach the apparatus and determine the direction of inserting electrode as shown in Fig. 3. The direction of inserting electrode is determined by measurement of the angle between the horizontal line (parallel to the base-line) and the line which passes through the aimed point and the trephined hole. It is very convenient if one puts the base-line on the rectangular sphere to the base-plate. Of course, the above described measurement is to be taken place by the help of x-ray or pneumoencephalogram.

We have designed the special electrode for recording EEG from the deep structure within the brain. It is a sort of the so-called multi-electrode. An operation consulting with thus recorded EEGs is particularly convenient

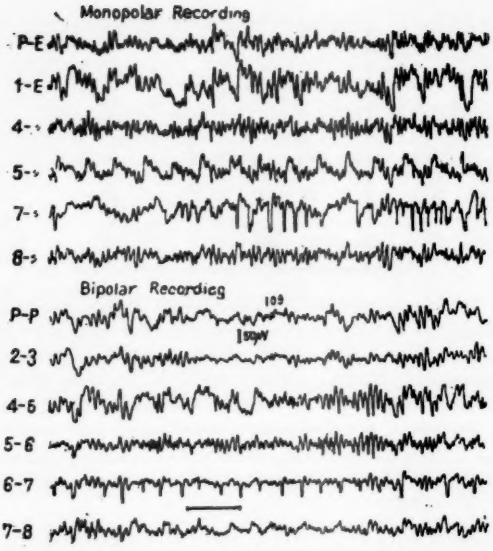

Fig. 4. A sample of electrogram recorded by means of the multi-electrode inserted into the brain and the stereotaxic apparatus. " E ": the left ear-lobe; " P ": the parietal region (scalp); " M ": the motor region (scalp); the number of active electrode: the position of subcortical one (" 1 ": 1 cm in depth; the same applies to those that follow).

The subject is idiocy (postencephalitic). In the 4th trace, a rhythmic large pattern superimposed many alpha waves is recognizable; this would perhaps originate from the brain pulsation within the brain-ventricle. The 5th trace has many positive spikes, however its nature seems difficult to discuss.

and worthy for determination of loci to add surgical manipulation, especially in cases of operations performed upon epileptics, as demonstrated in Figs. 4 and 5.

Summary

A new designed stereotaxic apparatus suitable for surgical manipulation of deep parts of the human brain was exhibited. And, at the same time, its modified points having some improvements were described with technical and operative notes.

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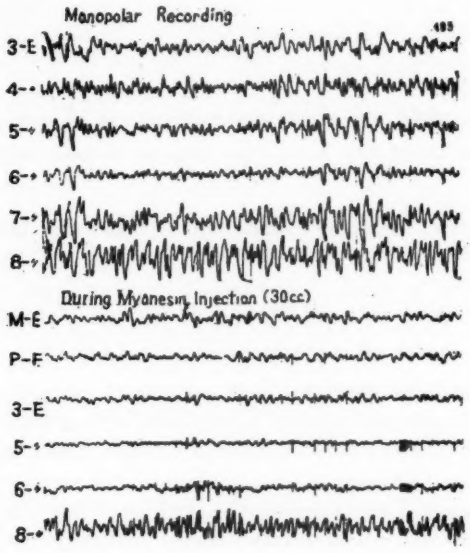

Fig. 5. Electrogram recorded from an epileptic patient with psychomotor seizure. The discharge from the deep part, or the thalamic one, is very dominant and irregular, and also seems being scarcely influenced by "myanesin." Such a view is rather routine and characteristic to the epileptics. When the difference between the cortical discharge and the thalamlic activity is dominant, it becomes easy to find out the focus necessary for determination on the basics of measurement.

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Studies on Mucopolysaccharide in Skin Diseases

I. Treatment of Urticaria Pigmentosa with Hyaluronidase

By

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(Received for publication, February 19, 1953)

Cases of urticaria pigmentosa, first described by Nettleschip in 1869, have been reported in 900 instances in Europe and the United States and in nearly 100 in Japan, since first reported by Tôyama in 1906, but the disease is as yet regarded as "rare." It is generally more prevalent by infants, but it may befall subjects of any age. As Unna has pointed out, an increase in the epidermal pigmentation and an infiltration of mastcells in the upper layer of corium are specific symptoms of importance, so that it is sometimes called mastcytoma or mastcellnaevus, but its pathogenesis and etiology are not yet clarified to date, some looking upon it as naevus or benign tumor, while others see reactive anomaly or reactive inflammatory variation in it. Consequently, no therapeutic method could claim sure efficacy against the disease. Recently, we have met with two cases of this disease, in which we found histologically, a large quantity of hyaluronic acid or heparin secreted by abundant mastcells, upon examination under utilization of Lison's metachromatic staining, so that we thought of trying administration of hyaluronidase, by which we have succeeded in bringing about a prompt and sure relief, as reported below.

Case 1. A male, age 10 months, first seen on Dec. 27, 1951, otherwise in good health and with no family history to mention. In the first part of Nov. 1951, he had disseminated yellow eruptions in size of millet seed on both legs, without any visible immediate occasion, which gave hardly any feeling of itching and disappeared in a week. In Dec. of the same year, pink-colored eruption of sizes varying from pin-head to Indian beans broke out on his left cheek and in a few days spread all over his body, causing vehement itches so that he complained of want of sleep.

Present status: Patient is a bottle fed infant, normal in growth and viscera, both liver and spleen impalpable and no swelling in lymphatic glands. The eruption is disseminated all over the body, except the haired

part of the head, being most conspicuous on the limbs. The most affected parts were the flexor surface of the legs and the upper arms, the back coming next, the cheeks and the side necks being also a little affected. The eruption consisted of light yellow-brown papules of sizes varying from pin-head to lentils, with distinct boundaries, elevated a little over the skin surface, flat-surfaced and of wax-like lustre, showing no diascopic discoloration. Its dermographia was positive, and by histamine scratch test, redness and swelling were tardier, both in appearing and fading, on the papules than on the normal skin, showing a lag in the time of reaction.

Laboratory examination: Blood Wassermann's reaction negative; red cell count, 4.2 millions; white cell count, 14,400; hemoglobin, 72% (Sahli); neutrophil leucocytes, 28%; eosinophil leucocytes, 2%; lymphocytes, 61.1%; monocytes, 9% and blood coagulation time, 6 min. 40 sec. All the above nomral. Phosphatide in serum lipoid, 204.48 mg%; free cholesterin, 137.70; total cholesterin, 162.18; esterized cholesterin, 24.48; neutral fat, 192.37; total lipoid, 574.21, which is a value larger than the Enickson-Williams standard.

Case 2. A male, age, 38, seen first on April 1951. Famliy history; late mother and brother had ichthyosis, the former having brown macules as seen by the patient. Patient married 7 years ago. Childless.

Past history: At the age of 16, had dermatitis occasioned by touching raw lacquer, and at the age of 27, suffered from toxicodermia upon eating sardines. Became conscious of yellowish brown macules of sizes varying from pin-head to half a grain of rice on his breast, when he was 29 years old, and sometimes felt slight itching. In the following year, the macules spread all over his body, growing especially conspicuous on his limbs and back. Upon warming himself or taking alcoholic drinks, the macules became markedly pigmented, and the itchy feeling grew stronger. At present, he complains of strong feeling of heart-burns and belching, upon feeding upon sardines and mackerel pikes.

Present status: Good nutrition and stature; viscera, normal; liver and spleen, impalpable; lymphatic glands in cervix and in cubital regions swollen to the size of Indian bean and millet seed respectively; eruption observed on the whole body except on the face, plams and soles, especially in large number on the extensor surface of the limbs. The eruption consists of typical brown macules, which are flush with the skin and readily redden upon rubbing, carrying up the pigmentary macules thereon in swells, blurring the boundaries of the pigmentary macules. In 20 min. or so, the redness disappears, restoring the distinctness of the pigmentary macules. Dermographia, positive; histamine scratch test shows retarding of redness and swell, both in developing and disappearing, recording a

lag in reaction time.

Laboratory examination: Mantoux's reaction, positive; blood sedimentation rate, 3 mm. (1 hr.) and 7 mm. (2 hrs.); blood Wassermann's reaction, negative; red cell count, 5.7 millions; hemoglobin, 82% (Sahli); white cell count, 7,200; lymphocytes, 34%; monocytes, 14%; eosinophil leucocytes, 8%; neutrohpil leucocytes, 44%; blood pressure, 120–64; Aschner's reaction, positive; pilocarpine test, positive; both adrenalin and atropine reaction negative; viz vagatonia presumable; Takada's reaction slightly negative and urin urobilinogen test positive, indicating functional disturbance of liver; phosphatide of serum lipoid, 151.94 mg%; free cholesterin, 57.12; total cholesterin, 129.54; esterized cholesterin, 72.42; neutral fat, 106.69 and total lipoid, 433.08, all approximately normal.

Histological observation: A pigmentary macule on the patient's lower region of right scapula was excised and stained with hematoxilin-eosin and under the method of Weigert. Also Lison's metachromatic staining of mucopolysaccharide was applied. The picture was decidedly typical, increase of pigment was conspicuous in the basal layer of the epidermis, and a multitude of mastcells was observed around the blood vessels in the upper layer thereof. The numerous granules of mastcells stained red, and by the scattering of the red granules upon addition of hyaluronidase to the section, existence of hyaluronic acid was assumed.

Therapy: In consideration of the histological observations above, the two subject patients were given injection of hyaluronidase (2,000 VUM) twice every week, either interdermally or subcutaneously in loco. The Case 1 lost the itching on the second day after the second injection, and began to sleep comfortably. The swell of the eruption began also to decrease gradually thereafter, some of the macules disappearing after the 6th injection, and when the 17th injection was given, the pigmentary macules almost entirely faded, leaving no itching at all. By the Case 2, the swells remarkably withdrew after the 5th injection, only a slight dermographia being left observable. With 15 injections the pigmentary macules faded visibly. Both the cases were judged cured to all intents.

Comment

The existence of proliferation of mastcells, the pathogenetic feature of the subject disease, is also the center of contention, some regarding the proliferation as the specific symptom of the disease, while others deny it. But the number of cases which showed a proliferation of mastcells is in an overwhelming majority, as shown in Hayami's statistics[1] on the incidence of this malady in Japan, which says that in his 77 cases of occurrence in total, only 2 were free of such a proliferation. Though mastcells were

believed to be generated by repetition of stimuli, in former times, their biological significance cannot be regarded as yet as clearly established. Recently, Asboe-Hansen[2] published his interpretation that " Mastcells are the producers of hyaluronic acid, and are interpreted as the peripheral transmitters of hormonal action on the connective tissue, perhaps by way of a precursor resembling heparin " and " Mastcells may also influence the hyaluronic acid-hyaluronidase system by the secretion of heparin." based upon a long series of observations, while Holmgren and Wilander, Luigi,[3] Leclércq,[4] Jorpers and others announced their opinion that the function of mastcells is the secretion of heparin and regarded them as heparinocytes. Prakken and Woerdeman,[5] in agreement with Battezatti, Hissard, Moncourier and Jacquet, assume that " mastcells have an improtant function in the metabolism of mucopolysacharides and perhaps, they may contain and release both heaprin and hyaluronic acid and eventually effect the metabolism of the latter into heparin," while Glick and Sylven contend that heparin is a hyaluronidase inhibitor. If mastcells secrete hyaluronic acid, the numerous red granules mentioned above in the histological observation must have been composed of hyaluronic acid. Recently, Degos, Hewitt and Morter[6] in Hospital Saint-Louis reported that no noticeable change was brought about by injection of hyaluronidase in the locally involved skin of an urticaria pigmentosa patient, but it may be pathogenetic interest that we succeeded in obtaining favorable results in two cases by repeated injection of hyaluronidase. The fact that our Case 2 had no child after 7 years in matrimony seems to suggest the problem of semen-hyaluronidase, and even the presumption that patients liable to urticaria pigmentosa are constitutionally under a specific influence of hyaluronidase. Furthermore, the occurrence of this rare disease in a family seems to indicate even an influence of hereditary genes on the varying predominance of hyaluronidase, if we borrow the physiogenetic expression of Goldschmidt.

Summary

As we had ascertained the existence of a quantity of hyaluronic acid in the histologically abundant mastcells of two cases of urticaria pigmentosa, by means of Lison's metachromatic staining, we applied injection of hyaluronidase in their involved skins interdermally or subcutaneously, and had the satisfaction of seeing both the cases cured to all intents. These results, we believe, may give some suggestions on the pathogenesis of this disease.

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An Attempt on the Intracardiac Phonocardiography

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Phonocardiogram is usually recorded from the chest wall but when such is picked up from the oesophagus, some components are obtained more clearly, such as the auricular sound.

A vibration that is perceived as the heart sounds is assumed to receive a certain amount of damping, such as resorption and reflection, on its way from the heart to the chest wall, and distorted to some extent. It seemed of physiological and clinical significance to pick up the prototype of the heart sounds from the oesophagus, or better still, from the cardiac cavity itself, rather than from the chest wall.

It would also be of clinical interest to obtain a phonocardiogram from various parts of the heart to which a catheter could be introduced. For this purpose, it would necessitate the use of a miniature microphone that could be attached to the tip of a catheter, but since such device was unavailable, following method was employed.

The method of a condenser microphone using the body as one pole, devised by Koizumi[1] and Hinohara[2] for picking up the heart sounds from the oesophagus, was adapted with some modifications. To the tip of the catheter or a plastic tubing, a metal stick, (a diagram of which is shown in Fig. 1) was attached and well insulated. Under a fluoroscopic observation, the tube was introduced into the heart cavity (to the dog) and this

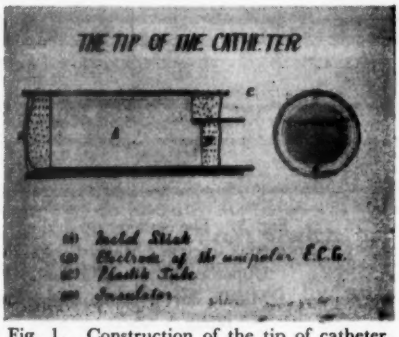

Fig. 1. Construction of the tip of catheter.

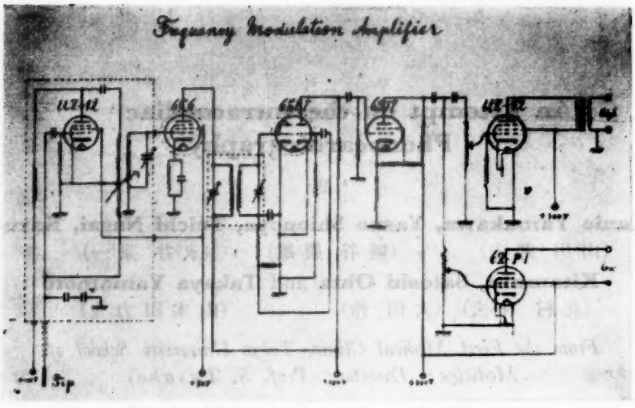

Fig. 2. Wiring diagram of our circuit.

was connected to the grid of an oscillator circuit shown in Fig. 2. It was assumed that the dielectric change that must occur by the vibration, which is thought to be the cause of the heart sounds, should then be transferred by frequency modulation to an oscillogram or be perceived directly as a sound through a loud-speaker.

The experiments were carried out on dogs and the oscillograms were taken with limb lead or intracardiac unipolar lead electrocardiograms. The recorded results are given in Figs. 3. By this means, a recording was obtained of a distinct vibration which occurred synchronously with the heart sounds that are usually recorded from the

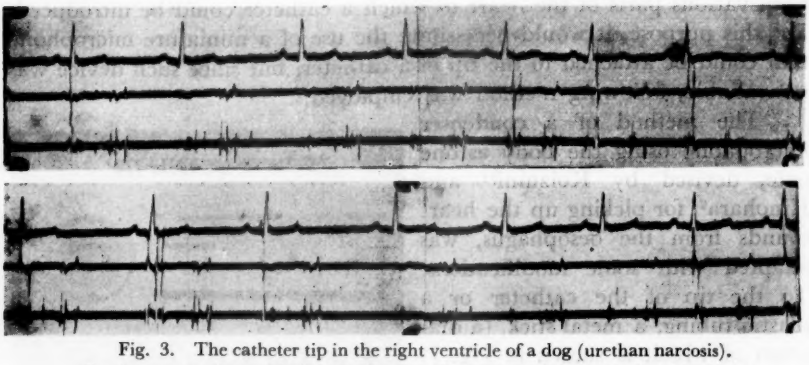

Fig. 3. The catheter tip in the right ventricle of a dog (urethan narcosis). Continuous tracing. Marked respiratory fluctuation of first sounds is shown.
Upper curve. Ecg. 2 lead.
Middle curve. Intracardiac Pcg. by "amplitudengetreue Abstimmung."
Lower curve. Intracardiac Pcg. by "gehörsähnliche Abstimmung."

chest wall as the auricular, first, second, and third sounds. Moreover, the heart sounds thereby obtained were found to possess the same characteristics as those of the heart sounds taken from the chest wall, i.e. the second sound is of higher frequency, is split, and has a vibration assumed to be due to the opening of atrioventricular valve, and the third sound is composed of vibrations of comparatively low frequency. The intracardiac auricular and first sounds were found to fluctuate with respiratory phases.

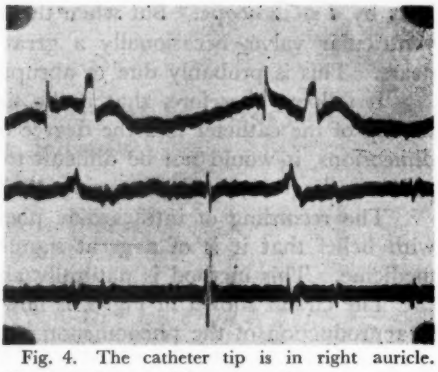

Fig. 4. The catheter tip is in right auricle. The auricular sound becomes evident.

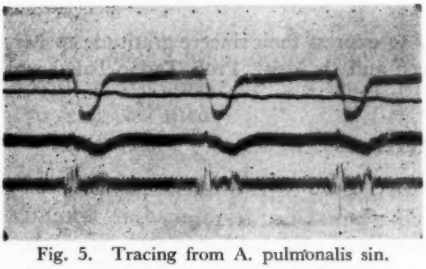

Fig. 5. Tracing from A. pulmonalis sin. The auricular and third sounds disappear.

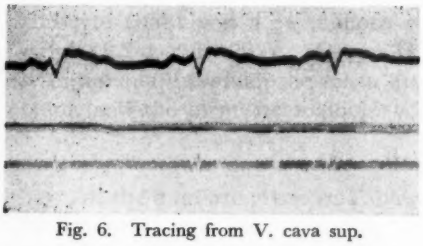

Fig. 6. Tracing from V. cava sup.

The phonocardiogram recorded from the chest wall during this stage did not give distinct tracings of auricular and third sounds. These sounds were obscure too on the tracing in A. pulmonalis. With equall amplification a trace of first sound was detected on the tracing in V. cava sup. When the amplification was too high, some mesosystolic vibration was detected besides the above in r. ventricle.

While such intracardiac catheter was introduced, the catheter was probably in frequent contact with inner wall and valve of the heart. However, no abnormal auscultative observations were detected. It was at first feared that some such artifact might appear on the intracardiac cardiogram by the introduction of the catheter into the heart cavity, but no such facts became evident when the degree of amplification was of a suitable dimension. The vibration that might be caused by such artifacts would probably be of a very low energy compared to the vibrations caused by the first and second sounds, therefore we could not

hear by a stethoscope. But when the tip of a catheter is near the atrioventricular valve, occasionally a great amplitude of the vibration appears. This is probably due to abrupt contact with the valve.

It follows, therefore, that if the size of the metal stick attached to the tip of the catheter and the degree of amplification were of a suitable dimensions, it would not be difficult to obtain an accurate intracardiac phonocardiogram, in the meaning of the prototype of the heart sounds.

The recording of intracardiac phonocardiogram is being continued with belief that it is of a great significance in physiology and clinical medicine. This method is naturally applicable to human beings.

The circuit shown in Fig. 2 is now being examined as to its fidelity in reproduction of the phenomenon.

SUMMARY

The intracardiac phonocardiography, in the meaning of the prototype of the heart sounds, was obtained by means of a condenser microphone using the body as one pole.

The authors take this opportunity to express their sincere gratitude to Mr. Tadashi Fukuda for technical assistance and to Assist. Prof. Tadao Takahashi for reviewing the manuscript.

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The Peroxidase Picture of Erythrocytes in Anemia

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In the present paper, I desire to report in which disease an appreciable change could if ever occur in the peroxidase picture of erythrocytes.

Experimental

Method

A modification of the counting chamber method of the copper peroxidase reaction (Sato and Shoji[1]) described by the author in a previous paper[2] was used.

Materials

The total number of cases examined was 133, and the majority were infants and children, 66 female and 67 male. For convenience' sake, the cases were divided into two groups, i.e. various diseases with (Group I) and without complication of anemia (Group II). Group I consisted of 80 cases of various diseases without anemia, in which the mean erythrocyte count was 4.84 millions per cmm. and the mean hemoglobin value 13,2 g. per 100 cc. Group II consisted of 53 cases of various cases with anemia, in which the mean erythrocyte count was 2.62 millions per cmm. and the mean hemoglobin value was 7.0 g. per 100 cc.

Results

All the data are given in Tables I and II, which are both summarized in Table III and Figs. 1 and 2. As is shown in Tables I and III, the peroxidase picture of erythrocytes in the cases of Group I was rather monotonous and uniform, nearly 90% of erythrocytes in each case were weakly peroxidase-positive and only a few per cent of ertythrocytes were strongly peroxidase-positive, although there were a few exceptional cases (Cases 22, 42, 61, 63, and 80). But in the cases of Group II, the per-

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TABLE I

Hematological Data and Peroxidase Reaction of Erythrocytes in Various Non-anemic Diseases

Case No.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill	HB g/dl	C.I.	Recticulocyte %	++ %	+ %	− %
1	H. N.	10y 6m	♂	10– 6–15	infectious hepatitis	360			1.0	0	99.8	0.2
				〃 – 6–21		420	12.6	1.0	0.1	0.6	99.4	0
				〃 – 6–29		400	12.0	1.0	0.4	0.1	99.8	0
2	S. S.	0y 4m	♀	49– 7–16	icterus (stenosis of bile duct)	400	11.0	0.9		2.0	98.0	0
3	S. S.	5y 0m	♀	50– 1–20	infectious hepatitis	560	14.5	0.85		0	99.9	0.1
4	T. H.	0y 2m	♂	49–12–17	congenital syphilis	450	13.8	1.0		5.6	94.4	0
5	S. K.	1y 1m	♂	48–12–27	meningismus	490				0.7	99.1	0.2
6	H. T.	11y 8m	♀	48612–27	epidemic meningitis	560				0.2	99.7	0.1
				49– 1– 4		540	17.9	1.11		0.6	99.4	0
7	R. N.	0y 4m	♀	49– 3–16	exanthema subitum	470	12.4	0.88	1.2	2.0	98.0	0
				〃 – 3–18		470	11.7	0.83	1.7	10.0	90.0	0
8	S. A.	0y 8m	♂	49– 2–21	acute diarrhea	480	10.3	0.71	0.9	1.2	98.8	0
				〃 – 3– 8		470	11.0	0.78	1.8	12.0	88.0	0
9	R. M.	30y		♀ 49– 2– 2	thromboartheriitis obliterans subclaviocarotica	590				4.4	95.6	0
10	I. I.	30y		♀ 49– 3– 1	sore throat	460	11.0	0.8	0.8	4.2	95.8	0
11	K. F.	0y 3m	♂	49– 3–19	pulmonary tuberculosis	560	11.7	0.7	3.0	19.3	80.7	0
12	T. M.	1y 5m	♂	49– 3–26	pleural empyema	400	11.5	0.95		3.4	96.6	0
13	Y. H.	6y 1m	♂	49– 1–29	acute glomerlonephritis	360	10.4	0.96		10.6	89.4	0
		6y 3m	♂	〃 – 3–16		405	13.5	1.13	3.0	6.0	94.0	0
14	M. F.	1y 9m	♂	49– 1–29	acute bronchitis	590	11.0	0.62		0.4	99.6	0
15	F. T.	5y 3m	♀	49– 3–10	bronchial asthma	480	14.5	1.01		1.4	98.6	0
16	A. T.	2y 6m	♀	49– 3–11	acute bronchitis	500	13.1	0.87	0.3	1.4	98.5	0.1
17	S. S.	1y 3m	♂	49– 3–22	measles pneumonia	550	11.7	0.71	1.1	12.1	87.7	0.2
				〃 – 3–24		600	14.5	0.81	0.7	0.8	99.2	0
18	M. K.	0y 1m	♀	49– 3–26	prematurity	710	23.5	1.10	0.2	3.5	96.5	0
19	R. S.	0y 6m	♂	49– 3–31	pertussis	410	15.2	1.23	2.4	0	99.8	0.2
20	K. S.	3y 4m	♀	49– 4– 2	pertussis	450	12.4	0.92	2.8	0	100.0	0
21	K. H.	6y 9m	♀	49– 4– 8	ascariasis	345	9.7	0.93	1.2	2.2	97.6	0.2
22	Y. K.	3y10m	♂	49– 4– 8	oxyuris and ascaris infestation with history of intestinal bleeding	500	13.8	0.92	0.1	59.4	25.2	15.4
						420	10.3	0.81	0.6	13.4	86.6	0
						500	14.5	0.97	0.6	0.3	99.6	0.1
23	S. K.	3y 3m	♀	49– 4–15	acute bronchitis	450	10·3	0.76	1.0	2.8	97.1	0.1
24	T. I.	0y 3m	♂	49– 4–15	congenital syphilis	560	20.7	1.22		6.9	93.0	0.1
25	H. H.	6y 2m	♂	49– 4–20	measles and pleurisy	400	15.2	1.27	0	160.	84.0	0

26	K. O.	1y10m	♂	49– 4–25	rhinitis	560	19.7	1.17		1.0	98.9	0.1
27	M. T.	7y 6m	♂	49– 5–11	measles	610	16	0.87		0.8	99.1	0.1
28	K. M.	3y 8m	♀	49– 5–17	measles-pertussis-pneumonia	420	13.5	1.07		4.4	94.3	1.3
29	M. I.	0y 2m	♀	49– 5–30	dyspepsia	560	19.5	1.16		0.5	99.3	0.2
30	G. O.	1y 0m	♀	49– 6– 1	thrombopenic purpura	525	12.3	0.78		0.2	99.8	0
31	T. M.	4y 6m	♀	49– 5–11	arrhythmia, heart murmurs	450	10.0	0.74		1.7	98.2	0.1
32	F. K.	0y 6m	♀	49– 2– 1	sore throst	500	13.1	0.87		1.6	98.4	0
33	F. E.	0y 8m	♂	49– 6– 6	Little's disease	490	14.5	1.02		0.2	99.8	0
34	S. I.	3y 5m	♀	49– 6– 6	tuberculous meningitis	540	11.5	0.7		0	100.0	0
		3y 9m		49–10–18		430	9	0.7		1	99.0	0
35	M. A.	0y 4m	♀	49– 6– 8	congenital syphilis	680	20	0.98		0.7	99.3	0
		0y 5m		〃 – 7– 2		447	14.5	1.08		2.2	97.8	0
36	F. S.	43y	♀	49– 6–20	Basedow	380	9.5	0.83		10.4	89.2	0.4
37	Y. A.	1y 8m	♂	49– 6–20	ascariasis	430	14.7	1.15		3.8	93.8	0.4
				〃 – 6–23		445	9.5	0.7		1.0	99.0	0
38	H. N.	0y11m	♂	49– 6–26	morbilloid rash	500	11.0	0.73		3.6	95.4	1.0
39	M. T.	1y 0m	♂	49– 6–23	bronchopneumonia	430	14.5	1.12		10.0	89.9	0.1
40	S. A.	0y 3m	♀	49– 6–27	erythrodermia desquamativa	340	14.5	1.42		29.0	91.0	0
41	K. N.	0y 5m	♂	49– 6–27	acute bronchitis	665	14.0	0.7		0.6	99.0	0.4
42	H. H.	0y 2m	♀	49– 6–28	congenital syphilis	440				86.4	13.6	0
43	M. T.	2y 9m	♀	49– 9– 5	pulmonary tuberculosis	400	10.0	0.83		0	100.0	0
44	M. H.	3y 0m	♂	49– 9–18	stomatitis aphthosa	360	11.5	1.06		0.2	99.5	0.3
45	F. O.	0y 9m	♀	49– 9–22	tonsillar hypertrophy	450	12.0	0.9	0.8	1.6	98.4	0
46	M. T.	1y 0m	♂	49– 4–18	sore throat	370			0.1	8.0	92.0	0
47	T. Y.	1y 1m	♀	49–12–18	thrombopenic purpura	425				0.1	99.9	0
48	Y. K.	3y 6m	♀	49– 9–11	acute colitis	420	11	0.88		0	100.0	0
49	A. K.	5y 8m	♂	49– 9–11	acute colitis	480				0	99.8	0.2
50	Y. A.	3y 8m	♀	49– 8–22	acute dyspepsy, severe	640				0.6	99.0	0.4
51	T. T.	1y 5m	♂	49– 8–22	bronchopneumonia	360				0	99.9	0.1
52	Y. S.	0y 4m	♂	49– 8–24	acute dyspepsy	440				2.4	97.4	0.2
				〃 – 8–28	whooping cough	400				0.2	99.6	0.2
53	K. I.	11y 8m	♂	50– 1–20		490	15.2	1.03		0.1	99.9	0
54	T. E.	1y 2m	♀	50– 3– 8	congenital syphilis	550				0.4	99.4	0.2
55	N. A.	1y11m	♂	50– 3–27	whooping cough	500	13	0.87		0.6	99.4	0
56	X. N.	0y 6m	♀	50– 4– 4	abdominal tumor	470	15.5	1.09	0.1	0.4	99.6	0
				〃 – 4–10		500	16.5	1.1		5.0	94.6	0.4
57	K. F.	0y 9m	♂	50– 4–15	congenital syphilis	550	11.0	0.66		0	99.8	0.2
58	H. M.	1y 1m	♂	50– 4–28	simple purpura	360				2.0	97.6	0
59	Z. Y.	10y 7m	♂	50– 5–24	acute rheumatic fever	445	11.2	0.84	0.4	0.8	99.0	0.2
				〃 – 5–31		440	13.0	1.0	3.6	0.6	99.2	0.2
				〃 – 7– 4		440	13.2	1.0	2.0	7.0	93.0	0
				〃 – 7–10		500	13.6	0.9	1.0	0.6	99.4	0
60	H. I.	0y 7m	♀	50– 7– 3	rickets, diarrhea	520	11.5	0.7		0	100.0	0
61	Y. K.	5y 3m	♂	50– 9– 1	severe dehydration, pellagra	510	14.5	0.9	1.0	30.0	69.9	0.1

Case No.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill	Hb g/dl	C.I.	Recticulo-cyte %	++ %	+ %	− %
63	Y. S.	0y 1m	♂	49–10– 2	congenital debility	550	17.0	1.0	0	3.0	97.0	0
				»–10–10		420				22.0	77.7	0.3
64	H. A.	0y10m	♀	49– 9–30	miliary tuberculosis	520	12.5	0.8	0.8	0.3	99.7	0
65	T. H.	1y10m	♀	49– 9–30	tuberculous meningitis	590			0.2	0.6	99.4	0
66	M.K.	2y 0m	♂	49– 9–30	acute bronchopneumonia	550	15.9	1.0	0.1	0.3	99.6	0.1
67	Y. A.	0y 6m	♀	49– 9–29	military tuberculosis	600	18.0	1.0		0.1	99.8	0.1
68	T. O.	1y 0m	♂	49– 9–26	scarlet fever	400	9.5	0.8		2.2	97.8	0
69	Y. I.	1y 6m	♂	49–10– 3	tuberculous meningitis	510				11.0	88.7	0.3
70	T. K.	1y 3m	♀	49–10– 4	pertussis, pneumonia	600	14.0	1.0		0.4	99.4	0.2
					leukemoid reaction	450				0.8	99.2	0
71	K. O.	7y 0m	♂	49–10– 8	idiocy	550	11.0	0.7		0.2	99.6	0.2
72	K. S.	4y 2m	♀	49–10–11	epidemic meningitis	400	11.0	0.9		0.2	99.8	0
				»–10–17		400	12.0	1.0		0	100.0	0
				»–10–20		430	14.0	1.1		0.5	99.6	0
73	S. O.	1y 2m	♂	49–10–18	pertussis pneumonia	570				0.5	99.4	0.1
74	T. S.	8y 1m	♀	49–10–18	acute colitis	570				0	100.0	0
75	K.W.	1y 0m	♂	49– 8– 9	diarrhea, with dehydration	550				1.2	98.8	0
				»– 8–18		715				0.4	99.4	0.2
				»– 8–28		620				0	99.9	0.1
76	R. H.	3y 4m	♀	49– 8–22	acute diarrhea	430				1.2	98.8	0
						370				4.0	95.3	0.7
77	H. T.	1y 6m	♂	49– 8– 4	acute bronchopneumonia	460				0	100.0	0
				»– 8–10		390				0.4	99.6	0
78	Z. O.	0y 7m	♂	49– 8–16	acute diarrhea	530				0.4	99.6	0
				»– 8–23		500				0	99.8	0.2
79	T. I.	2y10m	♀	49– 8– 5	acute diarrhea	400				0	99.8	0.2
				»– 8– 7		450				0.1	99.9	0
				»– 8– 9		500				0.4	99.6	0
				»– 8–14		430				0	100.0	0
				»– 8–20		370				1.0	98.8	0.2
				»– 8–23		460				8.0	91.7	0.3
80	H. K.	4y 2m	♂	49– 1–12	nephrosis	480	13.1	0.91		1.8	98.0	0.2
				»– 1–12		430	15.2	1.18		10.0	90.0	0
				»– 1–25		615	14.5	0.78		22.0	78.0	0
				»– 1–27		375	9.7	0.86		8.6	91.4	0
		4y 3m		»– 2– 2		415	13.8	1.11		2.7	97.3	0
				»– 3–13		450	10.3	0.76	4.1	15.5	97.5	7.0

TABLE II

Hematological Data and Peroxidase Reaction of Erythrocytes in Various Anemic Diseases

Case No.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill	Hb g/dl	C.I.	Reticulocyte %	++ %	+ %	− %
1	S. K.	46y	♂	49– 5–18	aplastic anemia	130	3.5	0.92	d.b. 0.2 p.b. 0	98.9	0.6	0.5
				„ – 5–20		120	4.0	1.11	d.b. 0.2 p.b. 0	92.0	7.7	0.3
2	B. R.	50y	♀	49–12– 5	lymphogranulomotosis, anchylostomiasis	270	5.5	1.08	1.5	35.0	65.0	0
3	K. K.	40y	♂	50– 6–25	cancer of stomach	300	6.5	0.72	8.2	0.3	99.6	0.1
4	K. O.	4y 9m	♀	49– 5–12	anchylostomiasis	300	5.5	0.61	d.b. 0.7 p.b. 0	3.9	85.3	10.8
5	M. G.	5y 2m	♂	49– 6–14	myxedema	430	8.5	0.65	d.b. 0 p.b. 0	5.5	94.5	0
6	A. Y.	1y 2m	♀	49– 7– 6	rickets, severe	360	7.0	0.65	d.b. 3.5 p.b. 1.4	50.2	49.8	0
7	T. S.	1y 0m	♂	50– 1– 9	splenomegaly, bronchopneumonia	400	8.3	0.7		2.4	97.6	0
8	H. K.	5y 0m	♂	50– 1–20	amebic dysentery	500	9.5	0.63		0.9	99.0	0.1
9	K. O.	1y 0m	♀	50– 7– 8	malnutrition, prematurity	450	6.3	0.5	0.4	13.2	86.8	0
10	H. T.	6y 1m	♀	50– 3–20	epistaxis, purpura	300	12.1	1.34		6.8	93.2	0
						330				5.0	95.0	0
						360				14.4	85.6	0
11	E. O.	10y 1m	♀	49– 9–27	pulmonary and internal tuberculosis	400	7.0	0.6	2.2	11.4	88.6	0
						370	9.0	0.8		9.0	88.7	2.3
						420	8.3	0.7		0	99.8	0.2
12	Y. W.	0y 6m	♀	49– 4–15	haemangioma cavenosum	330	9.0	0.9	3.8	30.0	69.9	0.1
13	M. K.	0y 2m	♂	49– 3–28	prematurity	340	11.0	1.08	9.2	40.4	58.6	1.0
14	T. S.	7y 8m	♀	49–10–17	hepatomegaly, anemia	340				0.6	99.4	0
15	J. Y.	0y 2m	♂	49–10–19	otitis media, anemia	400	9.0	0.75		37.0	62.4	0.6
16	M. M.	2y 1m	♀	49– 9–11	hepatomegaly, anemia	400	6.9	0.57		3.6	96.4	0
				„ – 9–14		350	6.3	0.6		6.0	93.6	0.4
17	H. M.	0y 2m	♀	48–12–24	congenital syphilis	280	11.0	1·31	d.b. 6.0 p.b. 3.1	33.6	66.4	0
18	M. K.	9y11m	♂	49– 3– 5	anchylostomiasis	280	7.0	0.83	d.b. 1.9 p.b. 0	42.2	51.6	6.2
				„ – 3–12		450	9.7	0.72	3.2	87.7	11.8	0.5
19	K. K.	7y 5m	♂	„ – 3–12	acute glomerulonephritis	390	8.3	0.71	0.2	7.2	92.8	0
20	U. M.	0y 2m	♀	49– 3–20	congenital megacolon	250	11.7	1.06	15.2	47.3	52.7	0
21	A. S.	16y 0m	♂	49– 3–23	backward boy, anemia	350	3.5	0.33	8.0	48.7	51.1	0.2
22	M. H.	5y 4m	♀	49– 3–24	ascariasis	310	11.3	1.21		8.0	91.6	0.4
23	M. H.	0y 4m	♂	50– 3–23	acute diarrhea	230			1.4	11.0	82.6	6.4
				„ – 3–24	blood transfusion reaction	270				28.6	70.9	0.5
				„ – 3–25		260	8.3	1.06	15.2	12.0	87.9	0.1

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				50– 3–26		290			10.2	25.0	75.0	0
24	A. S.	6y 0m	♂	51– 8–28	acute glomerulonephritis	280	5.5	0.65	0.5	95.0	5.0	0
25	T. W.	0y 2m	♀	49–10–22	congenital syphilis	280	8.0	0.95		0.4	82.0	17.6
26	S. K.	1y 1m	♀	50– 2–13	hypochromic anemia	390	6.5	0.55		0.1	99.0	0
				» – 2–19	(congenital?)	300	5.5	0.61		4.0	96.0	0
				» – 3– 7		300	5.9	0.66		2.2	97.6	0.2
27	M. N.	0y 2m	♂	50– 6–26	congenital syphilis	250	8.3	1.11	6.8	35.0	65.0	0
				» – 6–29		330	10.4	1.05	10.4	42.0	57.8	0.2
				» – 7–13		250	10.0	1.33	3.6	82.6	17.4	0
28	M. O.	0y 4m	♀	50– 5–28	atresia of bile duct	320	11.5	1.2	4.2	5.0	94.8	0.2
		0y 9m		» – 8–30	(confirmed by autopsy)	270	8.0	1.1	10.0	60.0	40.0	0
29	T. S.	7y 8m	♀	50– 5–23	infections hepatisis	330	11.0	1.1		1.0	99.0	0
30	Y. N.	1y 6m	♀	50– 2– 7	Gaucher's disease	300	6.0	0.66	d.b. 2.4 p.b. 0	31.0	68.6	0.4
				» – 2–13		350	9.7	0.99	21.0	1.6	98.4	0
				» – 2–17		280	7.2	0.85		2.0	98.0	0
31	M. T.	3y 4m	♀	50– 4– 6	splenomegaly, anemia	350			d.b. 1.0 p.b. 0	0.6	99.4	0
						360	9.7	0.9	1.8	1.4	98.4	0.2
32	N. S.	7y 5m	♂	50– 3–10	splenomegaly, anemia	190	5.2	0.9	1.2	52.6	47.3	0.1
				» – 4–13		230	5.4	0.8	2.2	34.8	65.2	0
				» – 4–14		240	4.5	0.6		22.0	77.2	0.8
33	S. K.	1y11m	♂	50– 4–25	hepatoma	390			1.0	20.0	80.0	0
				» – 5–29		270			2.0	0.2	99.8	0
				» – 6–20		260	6.9	0.9	0.4	7.6	92.2	0.2
				» – 6–27		160			0.6	26.0	73.4	0.6
34	K. M.	14y 9m	♂	49– 7–31	glycogen disease	270	8.0	1.0		2.0	98.0	0
				» – 8–13		240	8.0	1.0		9.3	90.7	0
35	H. N.	1y 9m	♂	49– 1–27	acute lymphatic leukemia	260	7.6	0.97	p.b. 0.1	50.1	49.9	0
36	T. K.	5y 0m	♂	49– 1–23	acute myelogenous leukemia	320	8.3	0.87	d.b. 0.1 Ebl. (+)	14.0	85.8	0.2
37	H. K.	41y	♀	49– 3– 2	acute lymphatic leukemia	190			25.0 Ebl. (+)	88.0	11.9	0.1
				» – 3– 7		100	3.5	1.15	7.0	87.9	12.0	0.1
38	T. K.	18y	♂	49– 3– 7	leucopenic myelogenous leukemia	120	3.5	0.96	0.6 Ebl. (+)	28.8	71.2	0
39	Y. Y.	29y	♂	49– 5– 1	myeloblastic leukemia	300	8.5	0.94		26.8	73.0	0.2
40	T. O.	40y	♀	49– 7–11	myelogenous leukemia	100	3.5	1.2	d.b. 0.5 p.b. 0	33.2	65.6	1.2
41	F. O.	51y	♂	49–11–10	myelogenous leukemia	100	3.0	1.0	2.3	31.4	68.6	0
				50– 1–20	,	120	3.3	0.91	1.7	97.4	2.6	0
				50– 1–27		240	4.5	0.62	d.b. 0.2 p.b. 0	37.0	62.9	0.1
42	K. S.	16y	♀	50– 3– 2	lymphatic leukemia	300	8.0	0.88	0.7	7.0	93.0	0
				» – 4– 3		120	5.5	1.57	1.6	39.4	60.6	0
43	A. G.	50y	♂	50– 3–16	myelogenous leukemia	200				6.0	94.0	0
				» – 4– 6		300	12.5	1.39		13.0	81.0	0
44	Y. K.	8y11m	♂	50– 4–27	lymphatic leukemia	190	5.5	0.97	0.1	29.0	71.0	0
				» – 5–29		250	9.0	1.20	3.3	3.2	96.8	0
45	Y. O.	3y 2m	♀	50– 5– 9	lymphatic leukemia	150	5.1	1.1	5.0	37.0	63.0	0
				» – 5–16		170	5.5	1.1	0	33.0	67.0	0

Case No.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill	Hb g/dl	C.I.	Reticulocyte %	++ %	+ %	− %
				50– 5–29		190	3.5	0.6		12.3	87.7	1
46	K. E.	21y	♂	50– 6–22	acute myelogenous leukemia	100	4.0	1.3	0	8.0	91.0	1.0
47	N. Y.	26y	♂	50– 8– 7	lymphatic leukemia	300	11.0	1.2	d.b. 0.1	0.8	99.2	0
48	M. I.	4y 8m	♀	50– 7– 5	acute lymphatic leukemia	120	3.5	1.0		78.6	21.4	0
				» – 7–10		115	4.1	1.16	1.0	74.4	24.8	0.8
				» – 7–12		120	4.0	1.11	1.0	57.8	42.2	0
49	K. K.	8y 0m	♀	50– 9–16	myeloblastic leukemia	80	4.1	1.7	3.0	55.0	43.0	2.0
50	Γ. T.	7y 2m	♀	50– 3–31	leucopenic melogenous leukemia	320	9.0	0.94	1.0	1.1	98.6	0.3
				» – 4– 1		340	9.6	0.94	0.2	5.0	95.0	0
				» – 4– 3		320	11.5	1.19	1.2	18.0	82.0	0
				» – 4– 5		360	11.3	1.04	1.4	0.4	99.5	0.1
51	H. H.	2y 9m	♀	50– 6–19	acute myelogenous leukemia	340	9.7	0.95	3.0	0.1	99.9	0
				» – 6–23		260	6.9	0.88	3.4	4.6	95.4	0
				» – 6–29		270	8.5	1.05	2.0	1.4	98.6	0
				» – 7–12		190	6.7	1.18		83.0	17.0	0
				» – 7–17		160	5.9	1.23		67.7	32.0	0.3
				» – 7–21		300	6.5	0.72	6.6	20.0	80.0	0
				» – 7–24		290	7.3	0.84		14.6	85.4	0
				» – 7–27		260	8.6	1.10	2.1	60.0	39.9	0.1
52	Y. K.	20y	♂	51– 3–14	refractory anemia: aplastic bone marrow, and miliary tuberculosis were confirmed at autopsy	117	3.7	1.05	1.8	90.2	9.6	0.2
				» – 3–16		110	3.7	1.12	1.8	45.0	54.6	0.4
				» – 3–18		124	4.1	1.11	3.6	53.6	46.4	0
				» – 3–20		102	3.4	1.10	2.4	47.0	52.6	0.4
				» – 3–22		98	3.7	1.2	1.2	60.0	37.4	2.6
				» – 3–25		100	3.4	1.15	2.4	58.0	40.6	1.4
				» – 3–29		105	4.1	1.3	2.2	46.0	54.0	0
53	H. K.	10y	♀	52– 3–20	aplastic anemia	72	3.0	1.3	0.8	42.0	57.5	0.5

Abbreviations: d. b. = diffuse basophilia of erythrocytes.
p. b. = punctate basophilia of erythrocytes.
Ebl. = erythroblasts.

oxidase picture of erythrocytes was greatly altered and, generally speaking, the peroxidase activity of the cells was remarkable, as will be seen from the very striking increase of strongly peroxidase-positive erythrocytes as was shown in Tables II and III. The degree of anemia and the increase of percentage of strongly peroxidase-positive erythrocytes did not always run parall to each other, it is true. It should, however, be kept in mind

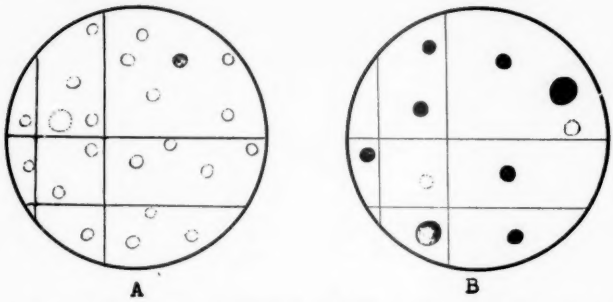

Fig. 1. Peroxidase pictures of erythrocytes in non-anemic (A) and anemic (B) case in a counting chamber. A) All but one in the right upper quadrant are weakly peroxidase-positive. One weakly peroxidase-positive neutrophil is seen in the left upper quadrant. B) Majority of erythrocytes and two neutrophils are strongly peroxidase-positive.

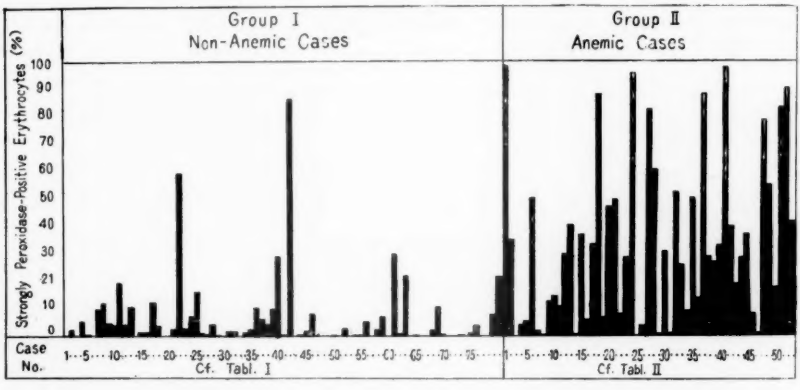

Fig. 2. The highest incidence of strongly peroxidase-positive erythrocytes in the blood of each case. The striking increase of strongly peroxidase-positive erythrocytes in percentage are seen mainly among cases of various kind of anemia (Group II).

that the alteration of peroxidase picture of erythrocytes might be connected with a number of different pathological factors causing the anemia.

Below I shall relate of a few cases among severe cases of anemia.

Iron-deficiency anemia: In two cases (Cases 3 and 4, Table II) the peroxidase reaction of erythrocyte was weak even before treatment, while in one case (Case 18, Table II) it had become much stronger during treatment.

Refractory or aplastic anemia; Three cases (Cases 1, 52 and 53,

Table II) were examined and a high percentage of strongly peroxidase-positive erythrocytes was revealed in each case. All of these three cases showed a marked depletion of cell elements in bone marrow.

Case 1 S. K. (Table II) died about one month after the examination, and the autopsy was not performed. Case 52 Y. K. (Table II) died about two months after the examination and the autopsy revealed miliary tuberculosis. Case 53 H. K. (Table II) was examined only once and unfortunately the follow-up study was not done.

Leukemic anemia: Seventeen cases (Cases 35 to 51, Table II) were examined, of which seven were of lymphatic and the other of myelogenous type. The strongly peroxidase-positive erythrocytes were of various percent. Two cases (Cases 47 and 50, Table II) showed no increase of the strongly peroxidase-positive erythrocytes, two other cases (Cases 36 and 43, Table II) showed a slight increase and the other cases showed a much more marked increase. In some case (Cases 41, 42, 44, 45 and 51, Table II) the strongly peroxidase-positive erythrocyte count fluctuated moderately in percentage during observation.

Miscellaneous anemia: In one fatal case of congenital atresia of bile duct (Case 28 Table II), strongly peroxidase-positive erythrocytes were once of 5%, but about one month later it rose up to 60% and on the next day the patient died.

Of three cases of anemia with splenomegaly of unknown etiology, two (Cases 7 and 31, Table II) had about 2% of strongly peroxidase-positive erythrocytes, while the other (Case 32, Table II) had 22～52,6% of them.

Of three cases of congenital syphilis, in which moderate anemia had developed, two cases (Cases 17 and 27, Table II) showed a high percentage of the strongly peroxidase-positive erythrocytes. In another case (Case 42, Table I) which showed a high percentage of such erythrocytes, hemoglobin value was not estimated but erythrocyte count was normal.

A striking increase (up to 50 to 95%) of strongly peroxidase-positive erythrocytes was seen in anemic cases of severe rickets (Case 6, Table II), congenital megacolon (Case 20, Table II) and acute glomerulonephritis (Case 24, Table II). A moderate increase (up to 20 to 35 %) was seen in cases of lymphogranulomatosis with complication of anchylostomiasis (Case 2, Table II), hemangioma cavernosum (Case 12, Table II), prematurity (Case 13, Table II), Otitis media (Case 15, Table II), idiocy (Case 21, Table II), transfusion reaction due to A B incompatibility (Case 23, Table II), Gaucher's disease (Case 30, Table II) and hepatoma (Case 33, Table II).

Comment

When I started this study, I expected that the peroxidase activity of erythrocytes might be increased in some cases of anemia. This expectation was based on the result of Minagawa's observation. Minagawa[4], reported that by the use of his modification of Sato and Shoji's counting chamber peroxidase method, the majority of nucleated red cells and some erythrocytes in the bloods of two cases of acute myelogenous leukemia and of two cases of anemia pseudoleukemia infantum were distinctly peroxidase-positive, while erythrocytes in the normal human blood were, as he stated, peroxidase-negative.

As already stated, under my own method, erythrocytes were weakly peroxidase-positive in cases of various diseases without anemia, while they were strongly peroxidase-positive in cases of various diseases with anemia. And thus, a great alteration of the enzymic activity of erythrocytes in anemia cases was confirmed. It is evident that peroxidase of erythrocytes may play some essential role in the blood of normal and pathological cases.

Most cases of anemia, in which the strongly peroxidase-positive erythrocytes increased, showed at the same time a reticulocytosis of various degree, suggesting that reticulocytes may have a higher concentration of this enzyme. And it was noticed that strongly peroxidase-positive erythrocytes occurred usually in a much larger number than reticulocytes, and so it seems to be certain that among strongly peroxidase-positive erythrocytes juvenile erythrocytes with no reticulum or substantia reticulofilamentosa may have been found. But it was also noticeable that strongly peroxidase-positive erythrocytes were in some instances (Cases 3, 23, 30, 31, 50, and 51, Table II) lower in number than reticulocytes, so that these cells were not always strongly peroxidase-positive.

Conclusions

Peroxidase reaction of erythrocytes was estimated by the use of a new technique of histochemical method (my own modification[2]) of the counting chamber method of copper peroxidase reaction) in 53 cases of anemia and 80 non-anemic cases. Needless to add that the present peroxidase picture of erythrocytes is entirely different from any peroxidase reaction performed on red cells on the blood smear.

Under this method, erythrocytes showed a various degree of the intensity of peroxidase reaction. In control cases, the peroxidase picture of erythrocytes was rather monotonous, and almost all the reds were weakly peroxidase-positive. In anemic cases, on the contrary, it was greatly altered, and the variety of intensity of peroxidase reaction of each

erythrocyte became very remarkable, and a large number of strongly peroxidase-positive erythrocytes occurred.

There was thus a striking difference of peroxidase activity of erythrocytes between anemic and non-anemic cases, though there were a few exceptional cases in either group.

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"Peroxidase Response" of Erythron in Anemia

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In a preceeding paper[1] of mine, I reported that the peroxidase activity of erythrocytes was greatly altered in cases of anemia and that alteration in these cases consisted mainly in a striking increase of strongly peroxidase-positive erythrocytes as revealed by a counting chamber method of peroxidase reaction devised by myself.

In the present paper, I desire to report a certain correlation between the hemopoiesis and the peroxidase activity of erythrocytes, on the basis of the result of serial examination of six cases of various kind of anemia and to discuss the hematological significance of this method.

Experimental

Method

The routine hematological procedures and the new technique of the counting chamber method[2] devised by myself were used.

Materials

A brief record of each case will be given below:

Case 1. T. K., 1 year and 3 months old, female. She was born one month prematurely and bottle-fed. She had developed hypochromic anemia that seemed to respond to iron. But she was discharged before the anemia was completely recovered.

Case 2. H. K., 22 years old, female. Case of idiopathic thrombocytopenic purpura. She had often purpural skin manifestation, and besides, at the time of menstruation, a large amount of blood loss causing a profound anemia, which was treated with multiple transfusion with improvement.

Case 3. E. K., a female nowborn. She was the first child of healthy parents, and born twelve days before term, weighing 2360 g. Delivery

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was normal. On the third day of life, she developed jaundice which gradually increased thereafter. On the thirteenth day, suddely she developed drowsiness and convulsions, and she was admitted to the University Hospital. For the following seven days these symptoms persisted, besides, she had fever, cyanosis, dyspnea occasionally simulating Cheyne-Stokes respiration and vomiting. At the onset, the examination of blood showed normal erythrocytes and hemoglobin level, though there was a macrocytosis, an increase of nucleated erythrocytes and an increase of icterus index. Anemia developed gradually within the following few weeks, and a splenomegaly ensued. Liver was palpable two finger breadth below the right costal margin on the day of admission. By an energetic and careful treatment, the patient improved and was discharged on the eightieth day of birth with sequlae of occasional mild attacks of convulsions resembling chorea minor and nodding spasm (suggesting kernikterus).

Subsequent tests of the blood groups of the members of the family showed that the father was of group O, Rh-positive, and the mother was of group AB, Rh-negative. And anti-Rh agglutinin was demonstrable in the mother's serum on the twentieth day after delivery. And the diagnosis of erythroblastosis fetalis due to Rh incompatibility was thus established.

Case 4. M. I., a 5 year 11 months old girl. She entered the Hospital because of bronchopneumonia. On admission, loud basal systolic murmurs and clubbed fingers, suggesting congenital heart malformation, were found. She was treated effectively treated with penicillin and discharged. In the next year she was readmitted because of meningeal signs and respiratory distressis. The diagnosis at this time was purulent meningitis, bronchopneumonia and glomerulonephritis. During the course of illness, massive intestinal hemorrhages occurred and moderate anemia developed. She was treated with penicillin, streptomycin and blood transfusions and improved.

Case 5. B. M., 36 year old man. A typical case of pernicious anemia showing a moderated, macrocytic anemia with megaloblastic bone marrow and achlorhydria and neurological symptoms, such as numbness and tingling of the hands and feet, for a few years. The course of illness was not influenced by iron treatment, but the patient responded well to vitamin B_{12}, while achlorhydria of gastric juice alone remained uninfluenced.

Case 6. J. K., 1 year old boy. He was admitted to the Hospital because of intermittent diarrhea, pallor and weakness. He was born at full term normally. During the pregnancy his mother was suffering from beriberi which was treated with thiamine. The weight at birth was about 3400 g. He was breast-fed, and for a few weeks prior to entry, a small amount of fruit juice had been given but no other food

up to the day of admission. He could not sit until the ninth month of age. There was no history of acute infections; and he had not been vaccinated yet. For about three months before admission, he had intermittent diarrhea and vomiting of milk, and he gradually developed pallor and weakness.

On admission, physical examination showed a moderately anemic, hypotrophic, and irritable infant weighing 8.25 kg. (normally 9.17 kg.) with enlarged liver as well as enlarged spleen (one and a half finger breadth below the costal margin). Cervical and inguinal lymph glands were enlarged. He had a slight ancle edema. There was no evidence of the involvement of the spinal cord and of visible mucous membrane. The acidity of gastric juice was within normal range. Blood showed a moderate macrocytic anemia with high color index and granulocytopenia with moderate hypersegmentation of nuclei of the neutrophiles. Bone marrow was megaloblastic. After entry, he was fed on mother's milk, cow's milk and fruit juice; thiamine, vitamin C and glucose were given intravenously every day. During the first two weeks of hospitalization, prior to starting any specific treatment for megaloblastic anemia, a marked reticulocytosis occurred unexpectedly and a gradual increase of erythrocyte count and hemoglobin level and granulocyte count followed, and simultaneously megaloblasts disappeared in the bone marrow. There was an incidence of bronchopneumonia about the end of the third hospital week, when vitamin B_{12} and folic acid were given for the purpose of preventing a possible recurrence of anemia and leucopenia, while penicillin also was given. The patient responded well and was discharged after recovery.

Results of Peroxidase Activity

Case 1 (Table I and Fig. 1). At first the strongly peroxidase-positive erythrocyte level was low in spite of the presence of a moderate hypochromic anemia, but it was temporally elevated by an iron medication.

Case 2 (Table I and Fig. 2). In this case a reciprocal relationship was seen between red cell count and hemoglobin value on one hand and the strongly peroxidase positive erythrocyte level on the other—quite an expected relation: when the former were low, the latter was high, and vice versa.

Case 3 (Table I and Fig. 3). In this case too, the reciprocal relationship between red cell count and hemoglobin value on one hand and the strongly peroxidase-positive erythrocyte level on the other was found. And an elevation of strongly peroxidase-positive erythrocyte level was found in a fair parallelism with that of reticulocyte level, suggesting a possible direct relation between a part of the strongly peroxidase-positive erythrocytes and reticulocytes.

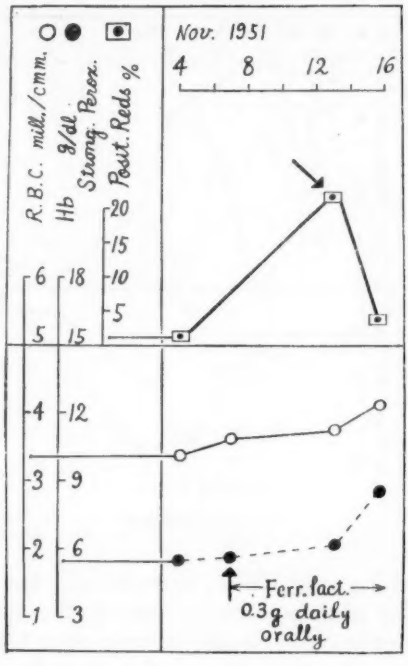
TABLE I

Case no.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill.	Hb g/dl	C.I.	Reticulocytes %	++ %	+ %	− %
1	T.K.	1y 3m	F	51–10–31	Iron deficiency anemia of bottle-fed infant	370	6.5	0.59	—	—	—	—
				〃–11– 4		335	5.5	0.55	—	0	99.8	0.2
				〃–11– 7		355	5.5	0.52	—	—	—	—
				〃–11–13		380	6.1	0.54	—	21.4	77.6	1.0
				〃–11–15		413	8.6	0.69		2.4	95.8	1.8
2	H.K.	22y	F	44– 1–11	Idiopathic thrombo-cytopenic purpura	400	11.7	0.98	—	11.0	98.8	0.2
				〃– 1–25		140	4.8	1.14	db. 1.2 p.b. 0.4	83.8	16.2	0
				〃– 1–29		210	4.8	0.76	d.b. 0.9 p.b. 0.4	77.8	22.2	0
				〃– 2– 5		300	4.4	0.49	d.b. 1.5 p.b. 1.1	38.0	61.2	0.8
				〃– 2–24		450	11.0	0.81	2.1	3.0	97.0	0
				〃– 3– 8		450	14.5	1.07	1.2	12.4	87.6	0
				〃– 3–30		350	12.4	1.18	0.9	1.0	98.0	1.0
				〃– 5–30		410	11.5	0.93	d.b. 0.1 p.b. 0	0.2	99.8	0
3	E.K.	13 days	F	50– 3–13	Erythroblastosis fetalis due to Rh incompatibility	650	18.0	1.03	0.7	—	—	—
		19		〃– 3–19		637	19.1	1.11	2.0	—	—	—
		23		〃– 3–23		566	15.6	1.02	1.2	—	—	—
		26		〃– 3–26		429	13.1	1.13	0.9	1.6	98.3	0.1
		34		〃– 4– 3		370	9.0	0.61	5.3	31.0	68.4	0.6
		38		〃– 4– 7		340	10.2	0.90	6.3	19.4	80.2	0.4
		42		〃– 4–11		345	8.7	0.8	7.5	34.0	64.8	1.2
		46		〃– 4–15		350	10.4	0.9	5.6	30.2	69.7	0.1
		52		〃– 4–21		335	11.0	1.0	5.7	40.0	59.8	0.2
		59		〃– 4–28		330	8.4	0.8	5.3	29.2	70.5	0.3
		68		〃– 5– 7		415			3.1	28.0	72.0	0
		78		〃– 5–11		460	11.9	0.8	2.6	10.0	89.8	0.2
4	M.I.	5y 11m	F	49– 7–11	Cong. heart disease	410	—	—	—	15.8	84.2	0
				〃– 7–16	Bronchopneumonia	350	—	—	—	5.2	94.6	0.2
				50– 4–17	Acute nephritis	230	4.5	0.65	6.0	56.0	44.0	0
				〃– 4–19	Intestinal bleeding	240	5.1	0.71	3.2	28.4	71.6	0
				〃– 4–21		270	6.2	0.77	6.0	57.7	42.2	0.1
				〃– 4–26		220	4.1	0.62	6.5	37.0	63.0	0
				〃– 4–29		220	5.0	0.76	9.0	57.6	42.4	0
				〃– 5– 2		300	5.8	0.64	2.4	31.0	69.0	0
				〃– 5– 4		290	8.3	0.95	1.8	35.0	65.0	0
				〃– 5– 7		290	7.5	0.86	0.4	67.0	33.0	0
				〃– 5–12		350	6.6	0.63	—	13.6	86.4	0
				〃– 5–18		300	4.7	0.52	4.6	21.0	79.0	0
				〃– 5–26		310	—	—	10.0	0.8	99.0	0.2
				〃– 5–31		—	6.5	—	—	—	—	—
				〃– 6–13		310	10.5	1.13	1.4	0.1	99.9	0

Abbreviations: d. b. diffuse basophilia of erythrocyte.
p. b. punctate basophilia of erythrocyte.
R.B.C. Red blood corpuscles.
C.I. Color index.

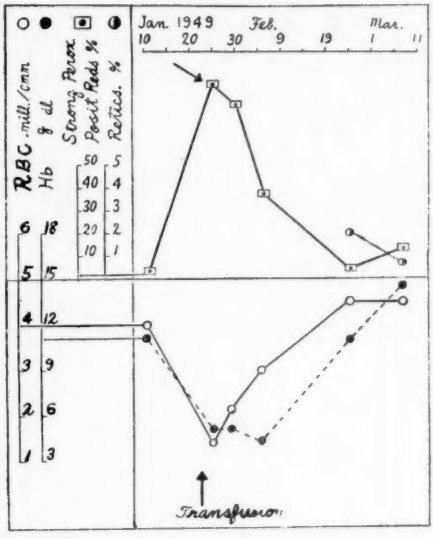
Case 4 (Table I and Fig. 4). This case showed rather eventful courses of erythrocyte count, hemoglobin value, reticulocyte count and strongly peroxidase-positive erythrocyte level, probably because of multiple complications of different clinical entities, and yet, a rough relationship between red cell count and hemoglobin value on one hand and strongly peroxidase-positive erythrocyte count on the other will be seen.

Fig. 1 (Case 1)



Case 5 (Tables II, III, and IV, Figs. 5, 6, 7 and 8). Neither clinical picture nor laboratory data including the peroxidase activity of erythrocytes were changed by iron treatment, but a great alteration was induced by vitamin B_{12}. At first, within five hours after the first intramuscular injection of 15γ of this vitamin the peroxidase activity of megaloblasts in the sternal marrow was remarkably increased, which is shown in Table IV as an increase of peroxidase-positive and even strongly peroxidase-positive cells of megaloblast series. This phenomenon was followed by a decrease of megaloblasts and an active proliferation of macroblasts, though the latter did not become evident until forty eight hours after the first vitamin B_{12} injection. The high peroxidase level of erythroblasts persisted about on week, during which time erythropoietic process had been restored to the normal way, and developmental and proliferative activity was highly stimulated. A fortnight later on, the peroxidase activity of normoblasts were found gradually decreasing to the normal level. (At the same time a small number of extracellular nuclei with high peroxidase content were visible). The strongly peroxidase-positive type of erythrocytes also began to increase in the bone marrow at the same time as that of nucleated erythrocytes, and they, both strongly peroxidase-positive and nucleated erythrocytes decreased again in successive stage. In the circulating blood, also, the strongly peroxidase-positive erythrocytes began to increase in number soon after vitamin B_{12} treatment was commenced and reached its

Fig. 2 (Case 2)



maximum on the seventh day of specific treatment, and then gradually decreased in number. As to the reticulocyte reaction it developed and reached its maximum on the fifth day of specific treatment and then subsided again. It was noted that the strongly peroxidase-positive erythrocyte count was always larger than the reticulocyte count, and that the high enzymic activity persisted more or less long after the reticulocytosis had disappeared. Erythrocytes and hemoglobin increased gradually but steadily and on the sixteenth day of the specific treatment they were 3.76 millions per cmm. and 11.3 g. per 100 cc. respectively. On the fourteenth and the twenty first day of the specific treatment, the peroxidase activity of bone marrow elements of granulocyte series were found to be much increased when the granulocytopoeitic activity was also hyperactive, showing a decrease of erythroid-myeloid ratio in the bone marrow and an increase of granulocyte count in the peripheral blood.

Some of megakaryocytes in the bone marrow were peroxidase-positive or/and even strongly peroxidase-positive in the second and the third week of the specific treatment, during which period platelet count in the blood had been more or less getting higher.

Case 6 (Tables V and VI and Fig. 9). In this case, the peroxidase activity of erythrocyte was very high on the second day of admission, and a few days later a reticulocyte response developed without any specific treatment, and a gradual increase of erythrocyte count and hemoglobin level followed. One month later, after an incidence of bronchopneumonia, his general conditions and blood picture were found much improved, and at that time both the strongly peroxidase-positive erythrocyte count and the reticulocyte count were lower than before.

Summary of Results and Discussion

The present paper reports the results of hematological investigations

esp. that of peroxidase activity by the new technique of counting chamber copper peroxidase reaction in six cases of anemia, i.e. one case of iron deficiency anemia of a weanling, one case of idiopathic thrombocytopenic purpura, one case of erythroblastosis fetalis due to Rh incompatibility, one case of anemia due to intestinal hemorrhage, one case of pernicious anemia and one infantile case of megaloblastic anemia. A certain regularity was noticed in the relationship between the peroxidase activity of erythrocyte and the hemopoietic activity of erythron. In Cases 2,3 and 4, during their erythrocyte counts and hemoglobin levels had been normal, and in deficiency anemia Cases 1 and 5, during the specific treatment had not been given, the peroxidase activity of erythrocytes was relatively low. But in all of the presented cases, the peroxidase activity of erythrocytes was greatly stimulated at the early stage of blood regeneration, and this was shown rather as a sign of blood regeneration. This phenomenon was found in a reciprocal relationship between the erythrocytes and hemoglobin level on one hand and the strongly peroxidasepositive erythrocyte count on the other in Cases 2,3 and 4; and it

Fig. 3 (Case 3)

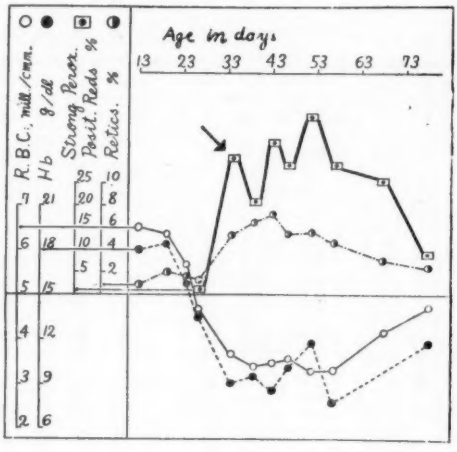


Fig. 4 (Case 4)

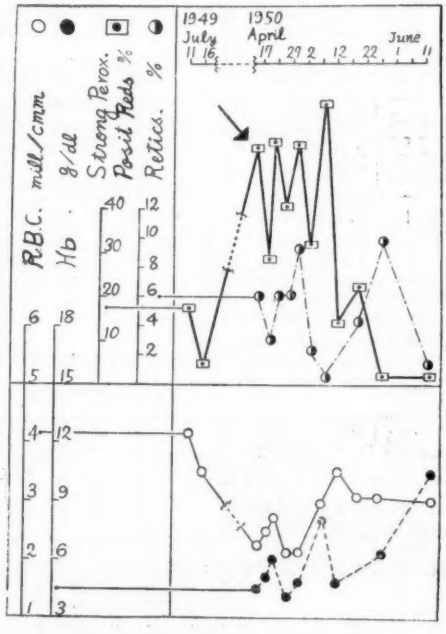


TABLE II

Date 1951	R.B.C. million	Hb g/dl	C.I.	Erythroblasts per 200 W.B.C.	Retics. %	Peroxidase activity of erythrocytes ++ %	+ %	− %	Platelets (thousands)	WBC	Differential count Eos. %	Eos. absol. count	Lym. %	Lym. absol. count	Mon. %	Mon. absol. count	Neut. %	Neut. absol. count	Neuclear shift of neutrophils I	II	III	IV	V	VI
Aug. 20	172	6.2	1.20	3	1.6	5.0	94.2	0.8	28.3	4200	5.0	210	15.2	640	5.8	240	74.0	3110	11	39	48	2	0	0
28	277	7.5	0.90		0.6	14.0	85.6	0.4	47.0	4200	4.4	180	27.5	1160	5.6	240	62.5	2620	5	28	36	28	3	0
Sept 3	210	6.3	1.00	4	0.7	5.4	94.2	0.4		4000	2.0	80	38.0	1520	3.0	120	52.0	2080	5	39	43	9	4	0
7	206	7.2	1.17	2	0.6	18.4	81.4	0.2	74.0	5800	3.0	120	43.0	1720	5.0	200	47.0	1880	6	26	43	19	4	2
10	190	8.3	1.46			13.0	87.0	0		4000	4.5	260	40.0	2320	6.5	380	49.0	2840	8	20	40	23	7	2
12	220	7.5	1.14			3.0	94.0	3.0			1.8	70	35.2	1410	3.0	120	60.0	2400	3	18	42	30	4	3
10:15 a.m.																								
3:15 p.m.	206	7.5	1.21			13.4	86.4	0.2	94.7	4100	3.0	120	51.0	2090	6.0	250	40.0	1640	2	18	30	44	2	4
13						30.5	69.4	0.1																
10:15 a.m.																								
14						23.0	77.0	0																
10:15 a.m.																								
15	219	7.6	1.16	1	3.0	35.4	64.5	0	70.0	4000	4.0	160	45.0	1800	1.0	40	50.0	2000	1	21	42	28	6	2
10:15 a.m.																								
17	267	8.3	1.04		7.4	61.0	39.0	0		5400	1.5	80	39.5	2130	10.5	570	48.5	2620	6	35	42	14	3	0
10:15 a.m.																								
19	315	9.2	0.97			65.0	35.0	0		4400	4.0	180	36.0	1580	12.0	530	48.0	2110	2	15	61	14	3	0
10:15 a.m.																								
21	300	9.0	1.0		3.5	8.0	87.6	4.4		5400	4.0	220	37.0	2000	11.0	590	48.0	2590	4	33	46	10	5	2
23	367	9.0	0.82		5.6	23.6	76.2	0.2	85.0	5900	2.0	120	38.0	2240	7.0	410	53.0	3130	6	28	36	22	6	2
25						31.4	67.6	1.0																
26	356	10.1	0.95		0.9	21.6	78.4	0	121.0	5600	3.9	220	30.6	1710	5.0	280	55.5	3110	4	22	44	26	6	0
28	376	11.3	1.00		6.9	12.4	87.6	0		7200	4.5	320	28.0	2020	5.0	360	62.5	4500	5	28	40	20	5	2
30						22.0	78.0	0																
Oct. 2						3.0	97.0	0																
3		10.8		2	1.0	37.4	62.6	0		7000	5.5	385	34.5	2420	8.0	560	42.0	2940	5	22	38	31	2	2
6					1.4	7.6	92.4	0																
8					1.0	4.0	96.0	0																
10					1.5	0.4	98.8	0.8	98.5	6600	0	0	29.0	1910	6.5	430	64.5	4260	5	45	39	9	2	0
12					0.6	0.6	99.4	0																
15					1.3	4.2	95.6	0.2																
17	400	11.0	0.92		0.3	0.8	99.2	0		6400	7.0	450	34.5	2210	5.5	350	53.0	3390	6	41	40	13	0	0
19					0.5	0.2	99.8	0																
22					1.1	0	98.6	1.4																
24	418	11.7	0.93		0.8	0	100	0	110.2	6600	3.0	200	31.5	2080	9.5	630	56.0	3700	7	40	38	13	2	0
26					1.1	0	100	0																
27	402	11.3	0.94		1.0	0	100	0		7000	4.0	280	38.0	2660	5.0	350	53.0	3710	8	34	43	13	2	0
31	401	11.5	0.96		0.7	0	100	0	96.0	7000	4.0	280	35.0	2450	5.0	350	56.0	3920	4	11	48	30	7	0
Nov. 8	392	12.1	1.03		0.7	0	100	0	98.0	6800	3.5	240	37.0	2520	4.5	310	55.0	3740	2	14	44	36	4	0
14	403	11.5	0.95		0.7	1.6	98.4	0	125.0	7000	3.0	210	35.5	2490	2.5	180	59.0	4130	10	46	34	10	0	0

TABLE III

Sternal Marrow Pictures of Case 5 B. M.

	1951 8–20	9–12 10:00 A.M.	" 3:00 P.M.	9–14 10:00 A.M.	9–19	9–26	10–10	10–24	10–31
Erythropoiesis									
Megaloblasts	8.4	13.4	10.0	2.8	2.6	1.8	—	—	—
Macroblasts	18.6	16.2	6.6	24.1	8.0	4.0	1.8	3.0	2.0
Normoblasts	39.0	12.8	14.6	24.1	25.0	11.6	7.4	9.4	10.2
Mitosis	0.6	1.0	0.4	1.5	0.8	0.4	—	0.2	0.2
Granulopoiesis									
Myeloblasts	0.6	0.4	0.6	0.3	0.2	1.6	0.2	2.0	0.8
Promyeloblasts	1.8	10.0	3.6	3.2	0.8	0.6	2.2	1.2	3.6
Myelocytes	8.8	18.8	16.0	1.5	10.0	9.8	8.2	4.4	6.4
Metamyelocytes	18.0	19.2	16.4	9.1	20.0	18.2	33.0	15.8	20.2
Segmented form	4.0	2.8	9.8	3.7	4.4	13.6	11.6	7.6	12.0
Eosinophiles	5.2	2.2	5.2	6.5	2.8	4.2	3.2	6.8	3.8
Basophiles	—	—	0.2	—	—	—	—	—	—
Monocytes	—	—	0.4	1.5	0.4	0.4	0.8	1.4	0.6
Mitosis	—	0.8	0.2	—	0.4	0.4	0.2	0.2	0.2
Reticulum cells and lymphocytes									
Reticulum cells	0.8	—	0.2	1.9	4.6	1.2	—	4.0	4.8
Lymphocytes	1.8	0.2	9.6	6.3	—	27.0	26.8	36.0	29.0
Mitosis of reticulum cells	0.6	0.4	0.6	—	0.2	0.2	—	0.4	0.4
Erythroid myeloid ratio	1.7/1	1/1.2	1/1.7	1.7/1	1/1.1	1/2.8	1/6.5	1/3.1	1/3.8

TABLE IV

Peroxidase Pictures of Bone Marrow, Case 5. M B.

Subject / Day of observation 1951	Peroxidase activity: Granulocyte series ++ %	+	−	Erythroblast series ++ %	+	−	Erythrocyte in marrow ++ %	+	−	Remarks
Sept. 12 10:00 A.M.	12.5	19.3	68.2	6.1	12.1	81.8	11.0	88.0	1.0	Sternal puncture was performed immediately before the first injection of B_{12}.
ditto 3:00 P.M.	10.9	21.1	68.0	32.3	32.3	35.3	28.0	71.8	0.2	Five hours after the first intramuscular injection of 15 γ of B_{12}.
Sept. 14 10:00 A.M.	13.5	25.1	60.7	14.9	42.4	42.7	31.4	68.6	0	
Sept. 19 10:00 A.M.	6.5	21.7	71.9	20.0	38.9	37.1	—	—	—	
Sept. 26	27.4	17.8	54.8	3.7	29.6	66.7	5.4	94.6	0	Strongly peroxidase-positive megakaryocytes were seen.
Oct. 3	36.6	27.3	36.1	11.9	27.0	61.1	16.4	83.4	0.2	Etracellular nuclei of erythroblasts were found either strongly peroxidase-positive or peroxidase-negative.
Oct. 10	11.7	33.0	55.3	5.5	17.2	77.3	1.6	98.2	0.2	
Oct. 24	15.6	26.0	58.4	0	8.7	91.3	0.2	99.2	0.6	

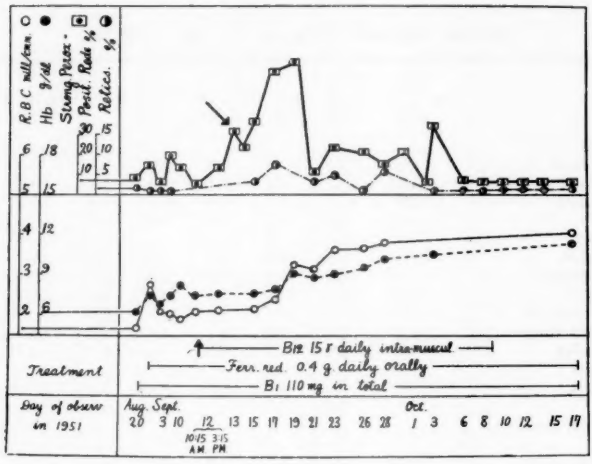


Fig. 5. (Case 5)
" Peroxidase Response " in the blood

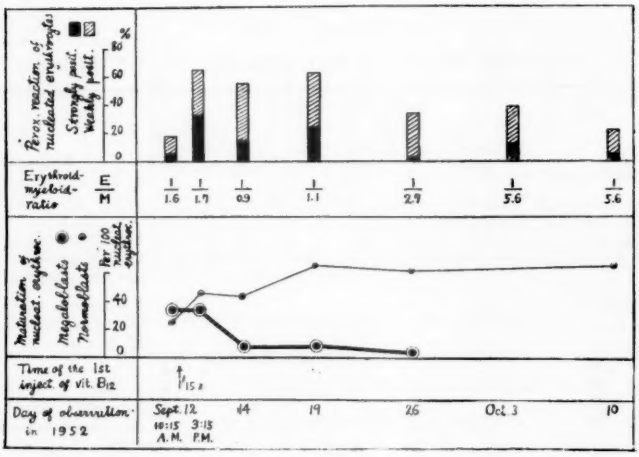
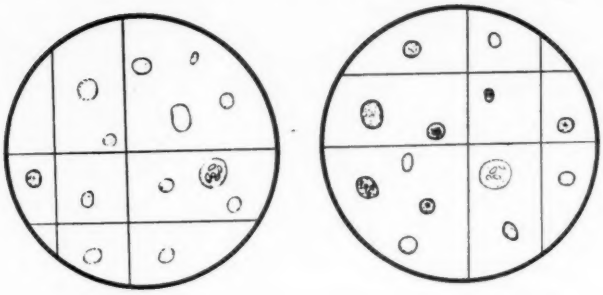


Fig. 6 (Case 6)
" Peroxidase Response " in the bone marrow

was found in a complete parallelism with the reticulocyte reaction in Cases 3 and 5.

Basing on the above described finding, I desire to suggest the name of " Peroxidase Response " to this phenomenon—the critical increase of peroxidase activity of erythrocytes in anemic cases. It is well known



A B

Fig. 7. " Peroxidase Response " of erythrocytes in the blood. (Case 5). A) Before specific treatment. B) On the third day of commencement of vitamin B_{12}. Note a critical increase of strongly peroxidase-positive erythrocytes.

that the reticulocyte response is the very earliest sign of blood regeneration. But the " Peroxidase Response " can, it seems, occur at a much earlier stage than the reticulocyte response, as will be seen from the results of my own cases. As described previously, in my own case of megaloblastic anemia (Case 6), in which an apparently natural remission had occurred, " Peroxidase Response " of erythrocytes seems to have occurred a few days earlier than the reticulocytosis which occurred later. In my own case of pernicious anemia (Case 5), which had been treated quite effectively with vitamin B_{12}, the restoration of the normal developmental and proliferative activity of megaloblasts was ushered in by a remarkable " Peroxidase Response " of the cytoplasm or/and nuclei of the cells, which had presented itself within five hours after the commencement of the specific therapy. The " Peroxidase Response " of erythrocyte in the blood of this case seems to have occurred at the same time as that of megaloblasts in the bone marrow, although the former was less evident than the latter.

The peroxidase reaction of granulocytes, mainly myelocytes and metamyelocytes, was also intensified in this case (Case 5), which was confirmed by serial examinations. (Even some megakaryocytes—usually peroxidase-negative—were peroxidase-positive.) So a kind of " Peroxidase Response " occurred thus in the granulocytic system, I believe.

Comment

Recently, interests of some investigators have been focussed upon the alterations of activity or/ and concentrations of enzymes in erythrocytes of various types of anemia. Sabine[3)4)] (1940) found that the cholinesterase activity of the erythrocytes was greater than normal in various

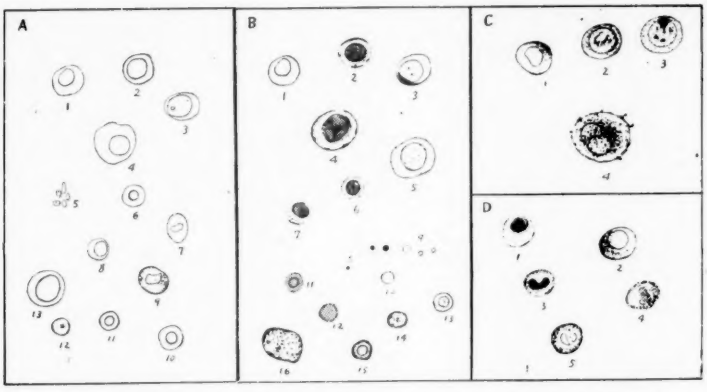


Fig. 8. " Peroxidase Response " in the sternal bone marrow (Case 5). A) Before the commencement of specific treatment. Majority of nucleated elements were peroxidase-negative. The cells 2,4,6,10,11 and 13 are peroxidase-negative, 7,8 and 9 are peroxidase-positive erythroblasts (of megaloblast series); the cells 7 and 8 seemed to be in mitosis. The cells 1 and 3 are peroxidase-negative myelocyte and myeloblast respectively. The cell 12 is weakly peroxidase-positive erythrocyte and the figures 5 are probably aggregated platelets, weakly peroxidase-positive. B) A remarkable " Peroxidase Response " of megaloblasts at the fifth hour of the commencement of specific treatment. Note the striking increase of peroxidase granules in nuclei and cytoplasm of erythron (2,4,6,7,14,15 and 16). The cells 5 and 13 are weakly peroxidase-positive megaloblast and normoblast respectively. The cells 1 and 3 are peroxidase-negative myelocyte and weakly peroxidase-positive promyelocyte respectively. The cells 11 and 12 are strongly peroxidase-positive erythrocytes with weakly peroxidase-positive Howell-Jolly's bodies ; the figures 8,9 and 10 are extracellular nuclei or nuclear remants, some of which were strongly peroxidase-positive . . . these were found in the second to fourth week of specific treatment. C) and D) a kind of " Peroxidase Response " of granulocytes and of megakaryocyte, seen in the second and the third week of specific treatment. Note the conspicuous increase of peroxidase granules in neutrophile myelocytes (C1, D1 and 2), promyelocytes (C2), myeloblast (C3), band neutrophils (D3, 4) and segmented neutrophils (D5). The cell C4 is juvenile megakaryocyte, strongly peroxidase-positive.

anemias; in pernicious anemia in relapse the enzymic activity was normal or low, and during treatment it rose to high values like thoses of other anemias, then fell gradually as the blood picture approached normal. The author stated that the relative concentration of enzyme in the red cells was a more sensitive indicator of hyperactive hematopoiesis than the reticulocyte and that failure of elevation of enzymic concentration in severe anemia was associated specifically with suppression or malfunction of the hematopoietic system. Valee[5] (1949) described that patients with

pernicious anemia showed no decrease in absolute values for zinc and carbonic anhydrase activity in spite of marked lowering of hematocrit and hemoglobin levels and erythrocyte count. Accordingly, zinc concetration and carbonic anhydrase activity per unit of blood were elevated, often to a marked degree. These increases were parallel, varying inversely with the degree of anemia; when they regressed under treatment, both did so at the same rate.

Kerppola[6] (1951) reported that, if there was accelerated regeneration of blood, alkaline phosphatase was then present in increased amounts —even in the hemoglobin-containing red blood cells—in a definite and regular manner; and that, cases of pernicious anemia without spontaneous reticulocytosis showed no appreciable phosphatase content in the bone marrow or in the peripheral blood. If such a patient was given liver extract, an increase of alkaline phosphatase, in connection with accelerated regeneration of blood appeared. The reaction was mild in some cases; in others it was fairly intense. The phenomenon was observed shortly before the appearance of reticulocytosis and was most pronounced at the stage when reticulocytes increase rapidly. This is what he described.

Now, I described in the present paper the phenomenon of " Peroxidase Response " of erythron in connection with stimulated erythropoiesis. These data seem to support the thesis that morphological evidences of erythropoiesis seem to be produced by underlying enzymic actions. Consequently it appears reasonable that the determination of enzymic activity of erythron provides a sensitive indicator of bone marrow function.

The phenomenon of " Peroxidase Response " of erythron was a favorable sign from the prognostic viewpoint at least in the presented anemic cases. But, as I reported in the preceeding paper,[1] high peroxidase levels of erythrocytes were found in various leukemic anemia too, and so it should be kept in mind that the prognostic significance of this phenomenon should be applied carefully and separately to each blood dyscrasia.

Remarks. The phenomenon of " Peroxidase Response " of erythron may forward a new problem to the study of anemia, though a further investigation and confirmation is necessary of course. In the present paper, " Peroxidase Response " of erythron is reported only preliminarily.

Summary

In order to evaluate a hematological significance of the peroxidase reaction of erythrocytes or/and erythroblasts, six cases of various type of anemia i.e. one case of iron deficiency anemia of a weanling, one case of idiopathic thrombocytopenic purpura, one case of erythroblastosis

TABLE V

Date 1950	R.B.C. mill.	Hb g/dl	C.I.	Ebl per 000 WBC	Retics. %	Peroxidase activity of erythrocytes ++ %	+ %	− %	Sedim. Rate	Plt. thousds.	Bleed. time minutes	WBC	Bas. %	Bas. absolt. count
Nov. 27	218	7.6	1.16	3	1.0				43(1hr.)	226	7.5	4850	0	0
28						91.3	8.7	0	71(2hrs.)					
Dec. 1	173	6.6	1.27	11	1.4							6700	0	0
4	198	6.3	1.06	38	14.4					156		7600	0	0
5	173	6.2	1.19		26.1									
6	258	7.7	0.99	30	38.1						6.0	7000	0	0
7	243	7.2	0.99		27.4									
9	348	8.0	0.77	10	22.8							5000	0.3	20
10	304	7.2	0.79		19.6									
12	380	9.0	0.79	4	10.3							6400	0.3	20
13	470	9.4	0.67		9.6									
14	466	10.1	0.72		5.9									
15	430	10.1	0.78	2	5.8					235	5.0	12000	0.5	60
16	415	10.1	0.80		7.1							20000	0.3	60
17	430	9.7	0.75	4	6.2						5.0	6100	0.8	50
18	410	10.4	0.85	2	9.4							7500	0.3	20
19	430	9.7	0.75	7	8.1							9600	1.0	100
20	435	10.4	0.80	14	8.2					224		20000	0	0
21	426	10.6	0.83											
22	390	9.7	0.85	5	9.0							7800	0.7	50
24	466	10.0	0.72							348	3.5			
25	498	10.7	0.72	5	7.2				4(1hr.)			7300	0	
27	536	10.7	0.67	5	2.8	23.0	77.0	0	8(2hrs.)	395		10500	1.3	140

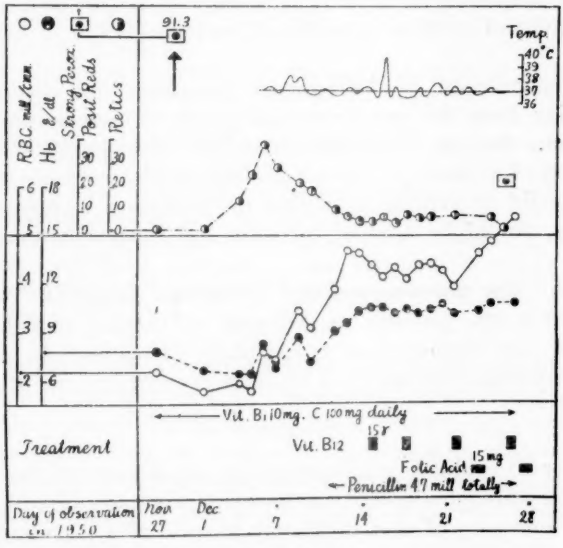


Fig. 9 (Case 6)

Differential Count										Nuclear shift of neutrophils						
Eos.		Lym.		Plasm.		Mon.		Neut.								
%	absolt. count	%	absolt. count	%	absolt. count	%	absolt. count	%	absolt. count	M.	I	II	III	IV	V	VI
0.5	20	75.5	3660	0	0	1.0	50	23.0	1120	0	2	36	38	22	2	0
0.7	50	66.5	4460	0	0	3.3	220	29.5	1980	0	3	26	41	20	8	2
2.0	150	61.5	4670	0	0	12.0	910	24.5	1860	0	6	25	50	9	8	2
0.3	20	63.5	4450	0	0	5.5	390	30.7	2150	3	3	16	45	18	11	4
2.7	140	35.0	1750	0	0	16.0	800	46.0	2300	1	5	23	34	24	11	2
1.3	80	35.0	2240	0	0	14.0	900	49.0	3140	0	7	31	41	15	5	2
0.5	60	43.0	5160	0	0	12.0	1440	44.0	5280	1	18	42	33	5	0	1
0	0	11.0	2200	0.3	60	13.4	2680	75.0	15000	2	31	42	21	3	0	1
0	0	28.2	1720	0.4	20	11.8	720	58.8	3590	1	14	48	32	4	1	0
0.3	20	53.3	4000	0.7	50	7.7	580	37.7	2830	2	21	49	23	4	0	1
1.3	120	45.0	4320	0.7	70	9.0	860	43.0	4130	0	18	43	36	3	0	0
1.7	340	70.3	14060	0.6	120	5.7	1140	21.7	4340	0	8	40	38	14	0	0
6.3	490	26.7	2080	1.0	80	10.3	800	55.0	4290	0	3	25	45	24	2	2
7.5	550	51.0	3720	0.5	40	6.0	440	35.0	2560	0	5	38	36	17	4	0
4.7	490	48.3	5070	0.3	30	7.0	740	38.3	4020	1	5	27	48	17	2	0

TABLE VI

Sternal Marrow Pictures of Case 6 J. K.

	1950 11–29	12–6	12–15	12–20	12–25
Erythropoiesis					
Megaloblasts	2.8	1.8	0.1	0.7	0.6
Macroblasts	8.4	1.5	0.6	0.8	1.8
Normoblasts	57.2	67.4	25.4	30.5	22.4
Mitosis	2.0	1.9	0.2	0.8	—
Granulopoiesis					
Myeloblasts	1.6	0.8	2.4	0.2	0.2
Promyelocytes	2.8	1.6	4.6	3.9	2.0
Myelocytes	10.1	5.5	9.0	17.3	6.8
Metamyelocytes	17.6	5.8	28.2	19.0	27.6
Segmented forms	10.8	10.1	22.4	18.2	28.6
Eosinophils	0.5	0.4	15.0	1.8	0.8
Basophils	0	0.1	0.6	0.3	0
Monocytes	0	0.8	0.4	0.1	1.4
Mitosis	0.2	0.2	0.2	0.5	0.2
Reticulum cells and lymphocyte reticulum cells					
Reticulm cells	0.5	0.4	0	0.4	1.6
Lymphocytes	0.8	2.6	5.4	5.5	6.0
Erythroid-myeloid ratio	2.5/1	2.5/1	1/2.6	1/1.8	1/2.8

fetalis due to Rh incompatibility, one case of anemia due to intestinal hemorrhage, one case of pernicious anemia and one infantile case of megaloblastic anemia, were observed under the serial examination by combined use of the new technique of counting chamber method of peroxidase reaction devised by the author and of other routine hematological procedures. The results may be summarized as follow;

1. A certain regularity was noticed in the relationship between the peroxidase activity of erythrocytes or/and erythroblasts on one hand and the hemopoietic activity of erythron on the other. The peroxidase activity of erythron was greatly stimulated, in other words the critical increase of strongly peroxidase-positive erythrocytes in blood and erythroblasts in bone marrow developed at an early stage of blood regeneration.

2. To describe this remarkable phenomenon—the striking increase of strongly peroxidase-positive erythron at an early stage of blood regeneration—the author suggested the designation " Peroxidase Response " of erythron.

3. " Peroxidase Response " of erythrocytes or/and erythroblasts was a favorable sign, for the course of anemia of the presented cases.

4 It was surprising how rapidly the phenomenon had occurred in a case of pernicious anemia, i.e. the " Peroxidase Response " of megaloblasts in the bone marrow developed within five hours of the commencement of the specific therapy, and that of erythrocytes in the blood was observed shortly before the appearance of reticulocytosis.

5. In an infantile case of megaloblastic anemia, a marked peroxidase response of erythrocytes in blood seemed to occur a few days earlier than the following reticulocyte response.

6. As had been shown in one of my own cases, the determination of peroxidase level of erythrocytes or/and erythroblasts may be useful as a bone marrow function test and consequently as a test of the potency of therapeutic agents for anemic cases.

Conclusions

The phenomenon of " Peroxidase Response " of erythrocytes or/and erythroblasts, i.e. a critical increase of strongly peroxidase-positive erythrons, was described as a sign of blood regneration on the basis of serial examination of 6 cases of various kind of anemia, including one case of pernicious anemia.

The " Peroxidase Response " was suggested for this striking increase of strongly peroxidase-positive erythrocytes in blood or/and erythroblasts in bone marrow in cases of anemia specifically treated. It could occur earlier than the reticulocyte response.

The counting chamber peroxidase method may thus provide a useful bone marrow function test, as well as a test of the potency of a therapeutic agent for anemic cases.

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On the AGogram of Blood Serum and Plasma

By

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Since November of 1949 we have been studying, by the use of Tiselius electrophoretic apparatus, the proteins of blood serum and plasma in a large number of cases of surgical diseases. In the course of the study difficulty arose as to the significance of the percentage and concentration of the protein fractions if merely their values themselves were referred to. Thus, it was felt necessary to find a suitable means of representation with regard to these fractions.

As regards visible blood components such as corpuscles, especially various white blood corpuscles, the ELMoNogram[1] devised by Emeritus Professor Akira Sato of our university proved to be efficient and valuable for interpreting the results of blood examination. Receiving a hint therefrom, attempts were made to apply this idea to invisible components of blood such as serum and plasma. Our diagram was first published at the general meeting of the Surgical Society of Japan, April 1951 under the name of "AGogram," taking the initials of albumin and globulin.[2,3]

Since then, our AGogram has been examined and criticized by several workers[4,5,6], some of whom prefer plasma to serum as the object of study, and others suggest to use percentage rather than concentration. Our preliminary but critical examination of these problems led us to adopt the concentration of protein fractions, as will be explained as follows:

If, in accordance with the original idea of the ELMoNogram, concentration is used, the total protein concentration and the percentage of each fraction can be readily calculated from the AGogram, whereas the AGogram with percentage has such a disadvantage as is analogous to that in the case of the ELMoNogram where only the percentage of each kind of white blood corpuscles is dealt with, regardless of their total number.

Method of drawing an AGogram. As shown in Fig. 1, four horizontal lines are drawn at equal intervals, representing albumin, α-, β- and γ-globulins respectively from above in the order given. A heavy vertical

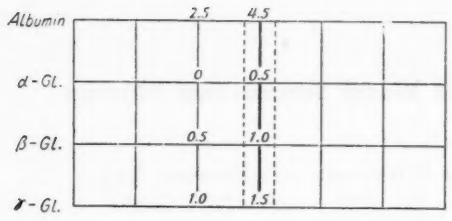

Fig. 1. Method of drawing an AGogram (serum).

line is then drawn in the middle, on both sides of which equi-distant, light vertical lines are drawn, thus many squares are formed by their intersection.

The intersection points of the four horizontal lines with the heavy vertical one represent the standard values of concentration of the respective fractions in normal persons. The average values of concentration of serum protein fractions as determined with 41 normal persons are shown in Table I. These results are on the whole in agreement with

TABLE I

Average Value of the Concentration of Total Protein and their Fractions in Normal Serum and Plasma

	Number of cases	Concentration of proteins (g/dl)					
		total protein concentration	albumin	globulin			
				α	β	φ	γ
Serum	41	7.3±0.79	4.43±0.50	0.49±0.07	0.92±0.07		1.42±0.15
Plasma	22	7.8±0.72	4.65±0.44	0.54±0.05	0.80±0.07	0.60±0.06	1.22±0.10

those obtained by several workers in this country. Taking simple numbers approximate to these values, the respective standard values of normal persons are 4.5 for albumin, 0.5 for α-globulin, 1.0 for β-globulin, and 1.5 for γ-globulin. Also one interval on the albumin line corresponds to 2.0 g/dl, and the same on the α-, β-, γ-globulin lines to 0.5 g/dl. It is also necessary to show clearly the range of fluctuation in each fraction of normal persons. Thus, based upon the fact that the range of fluctuation of albumin is 0.5 in normal persons, the normal range is represented by two dotted vertical lines drawn one on either side of the middle standard line, at an interval equal to 1/4 that of the square.

The blood plasma AGogram is drawn in the same way as the serum AGogram. The standard values of normal persons 4.5, 0.5, 1.0, 0.5 and 1.5, based upon the values obtained from 22 normal persons (Table I).

Type of the curve in an AGogram. In AGograms drawn for various diseases, there can be seen curves of various types, which may be roughly classified into the J- and reversed-S types.

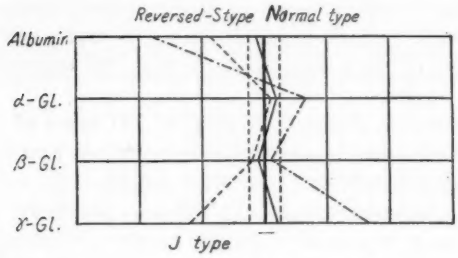

Fig. 2. Types of the curves in an AGogram.

When a curve lies within the two dotted vertical lines (Fig. 2), which represent the limits of fluctuation in normal persons, the case is taken to be normal. In the cases of both reversed-S and J-shaped curves, which extend beyond the normal limits, the curves indicate a decrease in albumin; the case of the reversed-S type is characterized by an increase in α- and γ-globulins, and that of the J-type by an increase in α-globulins and a decrease in γ-globulin.

To inspect grades in cases as represented by these three main types of curves the following angles are taken: in curves of the reversed-S type, the angle (A-γ) formed by the line connecting the albumin point and the γ-globulin with the albumin horizontal line (Fig. 3); and in curves of the J-type, the angle (α) that is formed by connecting the albumin, α- and γ-globulin points in order (Fig. 4). The angle A-γ thus defined

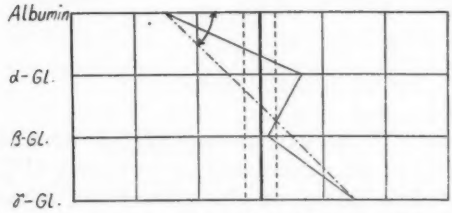

Fig. 3. Reversed-S type (angle A-γ) in serum AGogram.

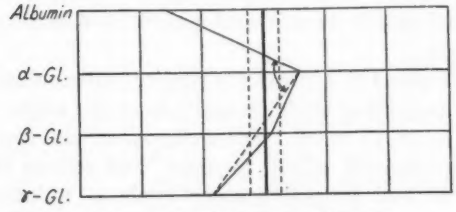

Fig. 4. J type (angle α) in serum AGogram.

varies in accordance with decrease in albumin and change in γ-globulin; and the angle with variations in albumin, α- and γ-globulin. The increase or decrease of each fraciton can, thus, be unitarily inspected by measuring these angles.

AGograms in various diseases. A total of 241 cases of various diseases were first divided into two large groups, tuberculous and non-tuberculous. The former was then subdivided into three groups of (1) renal tuberculosis, (2) pulmonary tuberculosis, and (3) others; similarly, the latter was subdivided into three groups of (1) gastric cancer, (2) gastric and duodenal ulcers, and (3) others. As will be seen from Table II, the great majority

TABLE II

AGograms in Various Surgical Diseases

	Disease	Serum			Plasma			sum
		normal type	reversed-S type	J type	normal type	reversed-S type	J type	
Tuberculous	renal tuberculosis	6	33	0	8	38	0	85
	pulmonary tuberculosis	4	3	1	6	8	0	22
	others	3	9	0	2	9	0	23
	sum (%)	13 (22)	45 (77)	1 (1)	16 (23)	55 (77)	0 (0)	130
Non-tuberculous	gastric cancer	5	8	50				63
	gastric & duodenal ulcer	6	3	12				21
	others	14	6	7				27
	sum (%)	25 (22)	17 (15)	69 (63)				111

of tuberculous cases, 100 out of 130 cases (77%), were of the reversed-S type, 29 cases (22%) of the normal and only one case (1%) of the J type. In the non-tuberculous group, on the other hand, a majority of cases, 69 out of 111 cases (63%), were of the J type, 25 cases (22%) of the normal and 17 cases (15%) of the reversed-S type. Especially noteworthy is that the curves of gastric cancer and gastric and duodenal ulcer cases were of the J type.

As shown in Table III, 131 cases of surgical tuberculosis were classified into three groups according to the magnitude of the angle A-γ: (1) below 50, (2) 51 to 70, and (3) 71 to 90. These cases were also divided, in another way, into three groups of: slight, medium, and serious illness according to local conditions and general findings such as pulmonary complication, blood sedimentation, body temperature. As for 62 cases of gastric

TABLE III

Reversed-S Type Angle A-γ in AGograms of Surgical Tuberculosis

Disease		Serum					Plasma				
		number of cases	average magnitude of angles	number of patients with A-γ angles			number of cases	average magnitude of angles	number of patients with A-γ angles		
				≦50°	51°～70°	71°～90°			≦50°	51°～70°	71°～90°
Renal tuberculosis	slight	7	80°		1	6	13	72°		1	12
	medium	11	71°	1	4	6	17	66°	3	8	6
	serious	21	58°	5	11	5	16	52°	7	9	
Pulmonary tuberculosis		8	87°			8	14	72°		5	
Others	slight	3	88°			3	1	71°			1
	medium	3	76°			3	5	75°		2	3
	serious	6	65°	1	4	1	5	49°	3	2	

TABLE IV

J Type Angle α in AGograms of Gastric Cancers and Ulcers

	Number of cases		Average magnitude of angles	Complications	Number of cases	Average magnitude of angles	Number of patients with α angles of		
							60°～100°	101°～140°	141°～190°
Cancer	without complication	18	109°				3	15	
	with complication	32	102°	pyloric stricture	8	105°	2	6	
				cancerous metastasis	13	103°	7	6	
				metastatic ascites	11	100°	6	5	
	sum	50	105°				18	32	
Ulcer	without complication	6	115°				0	6	
	with complication	6	93°	pyloric stricture	4	92°	3	1	
				ascites	2	94°	2	0	
	sum	12	104°				5	7	

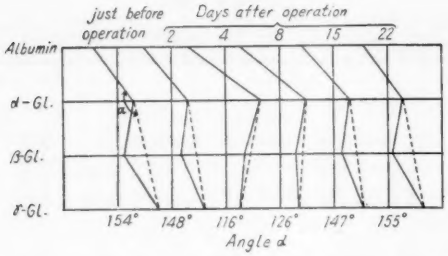

Fig. 5. An AGogram of thoracoplasty in pulmonary tuberculosis.

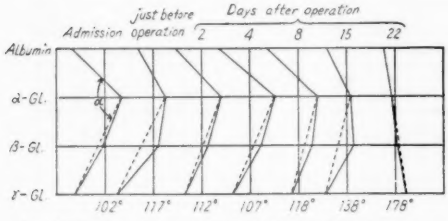

Fig. 6. An Agogram of gastrectomy in gastric cancer.

cancer and gastric and duodenal ulcers (Table IV), they were divided according to the angle α into three groups of 60 to 100, 101 to 140, and 141 to 180. Gastric cancer cases were subdivided into groups without complication and into groups with complications such as remarkable pyloric stricture, cancerous metastasis and metastatic ascites; and ulcer cases, into those of pyloric stricture and ascites deposit and these without complication.

Summarizing the results by referring to these classifications it has been concluded that the angle A-γ in tuberculous diseases and the angle α in gastric diseases become smaller in proportion to the extent of illness, that is, in accordance with seriousness and complicatedness of diseases, which agrees well with clinical symptoms.

Postoperative changes appearing in the AGogram. Of 101 cases of surgical tuberculosis, and 100 cases of gastric cancer and gastric and duodenal ulcers, the total protein concentration of blood serum or plasma and the content of the protein fractions were determined at the time of admission, just before operation, and 2, 4, 8, 15 and 22 days after operation, with the results as published in the Tohoku Igaku Zasshi.[7][8]

It will be understood that the variations in protein fractions are clear if the results are inspected on AGograms, which summarily represent these variations.

In Fig. 5 and 6 are shown two AGograms drawn for a representative

case of thoracoplasty in pulmonary tuberculosis and for that of gastrectomy in gastric cancer. As the postoperative variation in α-globulin is remarkable, the inspection of the postoperative variation is made by referring to the angle α.

It will be readily understood from each figure that, after the operation, the angle reduces until it attains to a minimal value in 4 to 8 days, then gradually recovers, returning to the value just before the operation or at the time of admission in 15 or 22 days respectively.

Summary

Herein is briefly described on the AGogram, a diagram showing the relations of protein fractions simply, which has been devised by us, following the idea of ELMoNogram (Sato's blood picture in regard to white corpuscles). Various curves of AGograms have been obtained in numerous surgical diseases, but they may be roughly classified into normal, J and reversed-S types. The J-type is often seen in the cases of gastric cancer, gastric and duodenal ulcers and the reversed-S type in the great majority of tuberculous cases. The significance of the angle A-γ in the reversed-S type and the angle α both in the reversed-S and J types are also described. In addition, some examples of AGograms are shown to demonstrate simply the postoperative change of the protein fractions.

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Cytodiagnosis of Malign Tumors by Means of Needle Biopsy and the Smear Method

Part I. Cytological Features of Hepatoma

By

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(Received for publication, March 23, 1953)

Since Kurokawa introduced the diagnosis by means of needle biopsy in 1940, this method has become the routine examination in our Clinic, sometimes bringing excellent results for conclusive diagnoses, even when other examinations are not sufficient. For microscopical examination of the acquired tissue, only histological preparations have been used, because of their reliability. Another method, the cytological study with smear preparations, can be carried out in a short time without employing any special instruments, but it has never been practically adopted, its accuracy being unreliable. This, however, can become one of the useful and convenient methods, if it is properly applied with due considerations given to its limitations.

On the other hand, tumor diagnosis by the smear method, particularly to distinguish carcinoma from sarcoma, or tell different kinds of tumors, has not yet been thoroughly investigated. Therefore, with a suggestion from Kurokawa I attempted a series of systematical observations of cytological features of neoplasmas in smear preparations. The preparations were made from the same materials as used for histological observations and were compared with the latter's findings. In this report are described the cytological features of hepatomas. While there are a good many papers on smear preparations of neoplasmas, descriptions on the special features of tumors are difficult to find in them, except those on the aquamous cell cancer by Stahel[1] and Tischendorf[2].

Experimental

Methods

As the needle biopsy for diagnosis in being practised in our Clinic, the materials furnished by it were used to get from three to five smears. Al-

though the smears were stained by several methods, only the observations about findings by May-Grünwald Giemsa stain will be found in this paper.

The diagnoses of tissues were carried out histologically with tissue sections, and the findings in smears were always compared with the histological ones. The materials, the histological preparations, together with their observations, were offered by the writer's co-workers, K. Mitsuki and T. Sato.

In needle biopsy, we endeavored to obtain not only cells floating in tissue fluid, but also tissue fragments, only to get smears. So that we employed a thick needle, 2 mm. wide in inside diameter, and a 20 ccm. record syringe. With the needle, successfully inserted into tissue, we could aspirate easily some tissue fragments each time.

For making smears, two technics were followed. When the tissue was tender, its small fragment was put on a slide glass with a small droplet of tissue fluid or blood, to be covered with a cover glass on it; the cover glass then was moved sideways with moderate pressuere of a thumb on it. If the tissue was tough, some tissue substance scraped from the fragment with an edge of the cover glass, was smeared upon the glass, together with some tissue fluid. In the smears of tissue fluid beautiful cell groups were found sometimes also. The smears were dried as quickly as possible, and stained.

Results

Liver cells: the tissue fragments of liver, obtained by needle biopsy, are slightly yellowish brown, and so tender that they can be smeared easily on the slide glass. Liver cells which are nearly normal are found, some separated individually, and others combined with each other. The combination of cells is relatively weak. The cells are almost round and their boundaries are considerably distinct outlined. Two-nuclear cells are often found. The cells are mostly 15 to 20 μ in diameter, while the two-nuclear cells sometimes reach to 30 μ. The nuclei are round and about 8 to 13 μ large, and rather coarse in its structure; they are very rich in chromatins and deeply stained. One or two nucleoli stained bluish are recognized relatively distinct. The cytoplasmic part is wide, well developed, and stained mildly basophilic, with a faint and very fine network recognized in it. Very often some granules which are assumed to be bile pigment are visible. They are apparently yellowish or bluish in color. In the case of jaundice these pigment granules are seen to be black and obviously larger both in size and number, and the cytoplasm is often stained acidophilic. Moreover, in the icteric liver many macrophages of large and small sizes are noticeable. They are mostly regarded to be Kupffer's stellate cells or endothelial cells, often greatly enlarged, con-

taining many pigment granules. In the case of the liver infiltrated by fat, many vacuoles are found in the intracellular and intercellular spaces.

Hepatoma cells: 10 cases of hepatoma, with their several characteristic features, are shown in Table I. The tissue fragments aspirated of hepa-

TABLE I

No.	Name	Combination of cells	Compression of cells	Arrangement of cells	Network in cytoplasm	Bile pigment granules	Fatty vacuolization
1	H. S.	weak	weak	rosette (+)	(+)	(+)	(−)
2	M. S.	relatively firm	weak	resembling hepatic cord	(−)	(−)	(−)
3	S. S.	weak	weak	rosette (+)	(+)	(−)	(+)
4	S. Y.	weak	weak	rosette (+)	(+)	(−)	(++)
5	R. E.	weak	weak	rosette (+)	(+)	(−)	(+)
6	M. E.	weak	weak	rosette (+)	(+)	(+)	(+)
7	G. Y.	weak	weak	rosette (+)	(+)	(−)	(++)
8	C. O.	weak	weak	rosette (+)	(+)	(−)	(++)
9	S. Y.	weak	weak	rosette (+)	(+)	(−)	(++)
10	F. K.	weak	weak	rosette (+)	(+)	(−)	(++)

toma are very often minute and floating in the blood with fine fat droplets, which a glance will suffice to recognize. But this is not always the case, for common tissue fragments are sometimes found, having no yellowish brown tone of the liver tissue. Generally, they are so tender that it is easy to smear them upon a glass. Microscopically, the combination of these cells with each other is usually more loose than that of liver cells, with only one case excepted so far. Therefore some of these cells are separated individually, while others are combined in small groups, often resembling a rosette in shape. In one case, the cells are combined relatively firm and arranged like a hepatic cord.

Hepatoma cells are generally much larger than liver cells, and remarkably polymorphous varying in size and shape. Nuclei are large, many of them being 10 to 20 μ, while the largest ones are about 25 μ. In two cases enormous giant cells, with enormous nuclei sometimes amounting to about 40 μ, are found. The shape of the nuclei is irregularly round, and their boundaries are not smooth. They are rich in chromatin and stained deeply. The chromatin is arranged in coarse aggregations and their boundaries are sharp. The nuclei usually have one or two nucleoli, which are in some cases very distinct and large, some of them as erythrocytes; in other cases, however, they are less distinct. Being pressed

by each other, nuclei in the cancer cells of other cancers often change their shape. But, in the case of hepatoma, the transformation of nuclei is slight.

The specific characters of hepatoma cells in smear preparations are mostly attributed to cytoplasm. When compared with other cancers, the cytoplasm of the hepatoma cells is well developed and large. It is basophilic and more sharply defined. In 8 cases out of ten hepatomas, fine network like that of liver cells is seen in cytoplasm. In two cases black granules, which are regarded to be bile pigment, are found. And in 8 cases many vacuoles large and small are remarkable in both cytoplasma and nuclei, and these vacuoles can be stained with sudan III.

Discussion and Conclusions

In ten cases of hepatoma observed on smears, hepatoma cells indicate not only the remarkable characteristics of malign cells, but also a considerable resemblance to liver cells. As for the former feature, there are nuclei two or three times as large as those of liver cells, with irregular shape, and with rich and deep stained chromatin, which are arranged in coarse and sharply defined aggregations, moreover the cytoplasm basophilic, and variety of the cells. As for the latter loose combination of the hepatoma cells and their mild transformation of nuclei, relatively wide cytoplasmic region, fine network and bile pigment granules sometimes visible in cytoplasm, and frequent appearance of marked fatty vacuolization, are observed.

Thus, by needle biopsy and with smear preparations, hepatoma is able to be easily diagnosed as a malign tumor, and relatively easy to be distinguished from other cancers. Especially this is true in the case of biopsy, for hepatoma to be distinguished from cholangioma or liver cancer transferred from stomach.

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Fig. 1

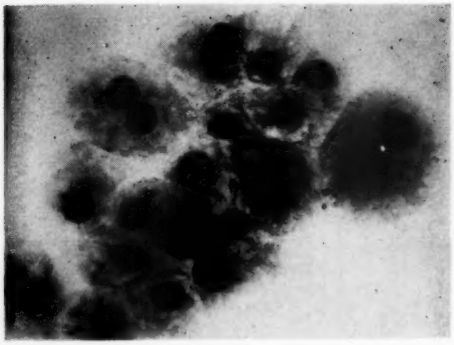

Fig. 2

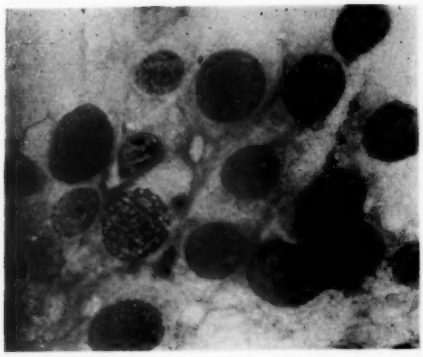

Fig. 3

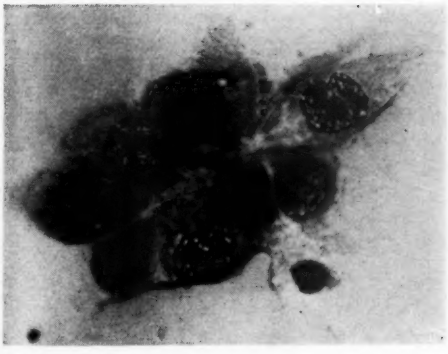

Fig. 4

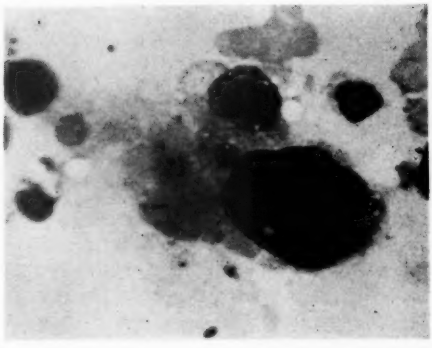

Fig. 1. Liver cells combined relatively loose. Out of them three are two nuclear cells. In wide cytoplasm fine network and a few bile pigment granules are found.

Fig. 2. The arrangement of cells in a hepatoma resembling hepatic cord. (Case No. 2)

Fig. 3. A rosette of hepatoma cells. (Case No. 2)

Fig. 4 and 5. Giant cells of hepatoms. (Fig. 4. Case No. 3, Fig. 5. Case No. 2)

Fig. 5

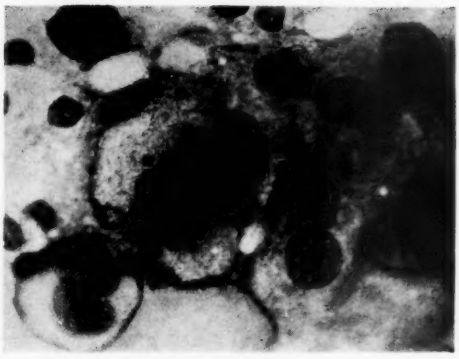

Fig. 6

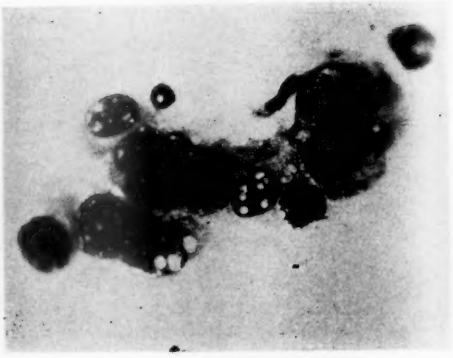

Fig. 7

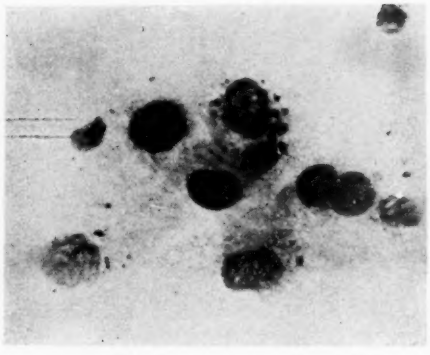

Fig. 8

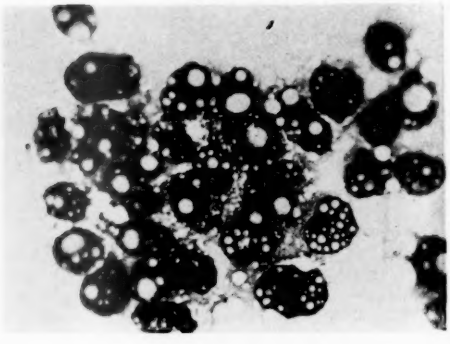

Fig. 6. Fine network in cytoplasma and mild vacuolization in hepatoma cells. (Case No. 7)

Fig. 7. A rosette of hepatoma cells and bile pigment granules in cytoplasm. (Case No. 6)

Fig. 8. Marked fatty vacuolization in hepatoma cells. (Case No. 9)

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On Nucleus Nervi Trochlearis in Human Adult

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(Received for publication, April 11, 1953)

In a previous research[1], I have made a minute microscopic study of the oculomotor nucleus in man, utilizing the Seto's silver impregnation in staining the sections. Following this, I proceeded to study the trochlear nucleus, using the same preparations, and arrived at the following results.

This nucleus lies in the upper part of the colliculus inferior, on the ventral side of the aquaeductus mesencephali and under the oculomotor nucleus (Fig. 1). In horizontal section, the outline is circular, a part jutting into the stratum griseum centrale, and another part into the fasciculus longitudinalis medialis. In sagittal section, it is seen as an elongated triangle. Pfeifer[2] gives the length as 1.5–2 mm. and the area of the horizontal section as 0.4 mm.^{2}, but in my specimens, I found a greater length of approximately 3 mm., and horizontal section area of 0.6 mm^2. The number of fibres, according to Merkel[3], is about 2.150. According to the studies of Sano[4] and Tsuchida[5], this nucleus is often seen spread out in sporadic cells, not forming a node. Sano asserts that the 11 cases that came under his observation were of the scattered type, of which 9 were scattered in both sides. In the three specimens I studied, one belonged to the unilateral scattered type.

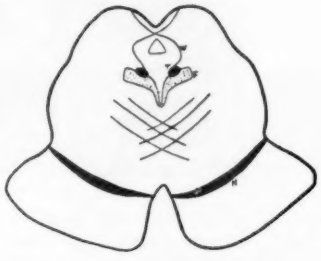

Fig. 1. Schema of horizontal section of adult human mesencephalon at the level of colliculus inferior. T nucl. n. trochl.; W trochlear root fibres, though it is impossible to represent their course on one horizontal section; F fasciculus long. medialis; N nucl. niger.

This is no difficulty in distinguishing the top part of the trochlear nucleus and the lower part of the oculomotor nucleus, since the former is round in shape, partially buried in the fasciculus longitudinalis medialis and sends out nerve fibres outwards, while the latter is rectangular in shape, closely touching out not penetrating the fasciculus longitudinalis

medialis, and sends out nerve fibres ventralwards. But no decision has been reached on the exact boundary of the both nuclei. Kölliker[6], Siemerling and Boedecker[7] assert transition and fusion of the two nuclei, while Bernheimer[8] speaks of a division between the two. According to Cassirer and Schiff[9], the two kinds of nuclei are sometimes connected by transition, sometimes bounded in a vugue way and sometimes, there is a zone devoid of cells between the two. In my 3 specimens, one had a cellfree zone and the other two were indistinctly boundaried. This fact seems to endorse the assertion of Cassirer and Schiff.

The nerve cells composing the trochlear nucleus can be classified into the major and minor cells. The former account for the majority, the minor cells being but few in number. The microscopic structure of the two kinds of cells is similar to those I have reported on in the oculomotor nucleus. The larger cells are provided with many stout processes and

Fig. 2

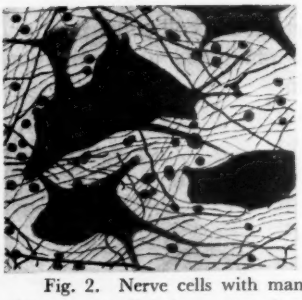

Fig. 3

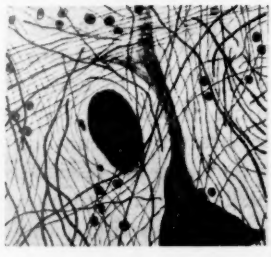

Fig. 2. Nerve cells with many stout processes and intranuclear nerve plexus in the glial tissue of nucl. n. trochl., sagittal section of adult human mesencephalon. Thick fibres are motor and sensory, thin fibres are vegetative. Seto's impregnation. X 300, reduced to 2/3.

Fig. 3. A small vegetative cell (middle) and a large motor cell (right lower) in nucl. n. trochl., in the same section. Intranuclear Plexus the same as in Fig. 2. Same staining. X 500, reduced to 2/3.

are virile in appearance (Fig. 2), while the smaller cells are about $\frac{1}{2}$ to 1/3 in size of the former, ellipsoid or pear-formed in shape, unipolar or bipolar and have rather oversized cell nucleus (Fig. 3). The former belong to the motor nerve cells and the latter to the vegetative, as they are in the oculomotor nucleus.

As described above, this nucleus n. trochlearis, similar to the oculomotor nucleus, which is functionally almost analogous to this nucleus, is composed of numerous motor cells and a smaller number of vegetative nerve cells. No sensory nerve cells, as have been found in the oculomotor nucleus, were ever observed, but interestingly enough, some sensory nerve cells of the most ventral part of the nucleus mesencephallicus nervi trigemini

were found in close proximity of the most dorsal part of the nucleus nervi trochlearis.

The course of the fibres of the trochlear nerve root in the mesencephalon is rather complicated, owing to the low position of the exit of the root fibres from the mesencephalon as peripheral nerve, in comparison with the level of the nucleus. The fibre bundle describes a horseshoe curve in its course, forming three legs through two geniculate bends. That is, the root fibres, emerging from the dorsal and outer side of the nucleus, first run horizontally extra-dorsalward to the outer side of the central part of the trigeminal root fibres (crus originis), gather into a bundle there, then turning a near rectangle, descend along the aquaeductus mesencephali a little on the dorsal side, making a geniculate curve, down to the vicinity of the vellum medullare anterius in the lower part of the colliculus inferior (crus descendens), bend in a horizontal plane inward toward the median line, intercross with the bundle from the opposite side on the median line, penetrate in the dorsal part of the aquaeductus mesencephali, and then run out of the brain in the vicinity of the curs cerebello-cerebrale.

The above described course has been accepted widely, but in addition thereto, I could observe a new course for the fibres originating in the lowest part of the nucleus, which form a small bundle upon emerging and ascending cerebrally, join the above mentioned crus descendens, a discovery particularly well evidenced in my sagittal preparations, as in Fig. 4. Thus it has been proven that the trochlear root fibres include not only the hitherto accepted descending bundle, but also an ascending bundle originated in the most basal part of the trochlear nucleus. I propose to call this tiny bundle crus ascendens.

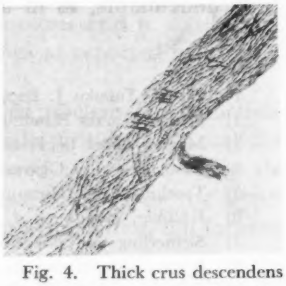

Fig. 4. Thick crus descendens and thin crus ascendens of my discovery joining it, coming from nucl. n. trochl., in the same section. Same staining. X 800, reduced to 2/3.

My thorough investigation has revealed the existence of trochlear root fibres decussating at the median line, but no others, such as non-decussating fibres or double-crossing fibres, as mentioned by Obersteiner[10] and Panegrossi[11], nor could I observe any indication of direct connection of the nuclei of both sides.

In conclusion, I will point out that the course of entrance of the motor nerve fibres from the tractus pyramidalis and the sensory nerve fibres concerned with the ocular reflex into this nucleus is extremely obscure, as has been the case with the oculomotor nucleus. However, the formation of very complex intranuclear plexus composed of thick and thin

fibres in the nucleus has been demonstrated, similar to that in the oculomotor nucleus, the thick fibres undoubtedly being motor and sensory, the thin fibres vegetative, in nature. How it comes about that the course of the incoming nerve fibres is yet in the dark, in spite of the clearly established formation of plexus in the nucleus, is an important question awaiting future solution.

Summary

Nucleus n. trochlearis, like the motor nuclei of nucleus n. oculomotorii, consists of a majority of large motor cells with many stout processes and a minority of unipolar or bipolar vegetative nerve cells.

The trochlear root fibres comprise beside the hitherto known crus descendens also my so-called crus ascendens, originating in the lower part of the nucleus. The root fibres always decussate at the median line without exception, no non-decussating or double-crossing fibres being found, nor any sight of direct connection between the nuclei on the both sides.

In the nucleus n. trochlearis is found, as in the oculomotor nucleus, nerve plexus formed of thick fibres of motor and sensory nature and thin vegetative fibres, but the course of these incoming nerve elements is utterly undefinable, as in the oculomotor nucleus.

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Nucleus Mesencephalicus Nervi Trigemini in Human Adult

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(Received for publication, April 11, 1953)

Nucleus mesencephalicus n. trigemini is yet a mooted subject, both histologically and physiologically. While Forel[1], Held[2], Kölliker[3], Kljatschkin[4], Terterjanz[5], Mahaim[6], Grunstein-Gurwitsch[7], Wollenberg[8] and Igarashi[9] assert its motor or centrifugal nature, Krause[10], Johnston[11], May-Horseley[12], Kosaka[13], Kappers[14] and Sherrington[15] deem it sensory in nature. With a view of elucidating this problem, we made a thoroughgoing investigation of the nucleus mesencephalicus and the nerve fibres connected thereto, using the same preparations as used by Atsuki[16][17] in his studies of nuclei n. oculomotorii and n. trochlearis, which were rewarded with many new observations as reported previously, and arrived at many new views as follows.

This nucleus represents what has been called nucleus aquaeductialis trigemini by Ziehen[18] and nucleus mesencephalo-protuberantia cellulo-vesiculosus by Nicolesco[19]. Its cells are arranged in an arc along the outer edge of the stratum griseum centrale, extending from the upper part of the pons to the colliculus rostralis, without aggregating, but forming small groups or scattered over the area.

The nucleus is composed of many special cells and a small number of vegetative cells. The former, as shown in Figs. 1 and 2, are entirely dissimilar to the motor cells found in the nuclei n. oculomotorii and n. trochlearis, being conspicuous in their globular or ellipsoid feminine appearance. Their size has been estimated at 45–60 μ by Obersteiner[20], 40–80 μ by Kölliker[3], and in our specimens, at 30–80 μ.

Whether these cells are provided with processes or not has been yet an unsettled question. According to Golgi[21] and Johnston[11] they are unipolar, according to Krause[10] and Merkel[22] they are bipolar and according to Kölliker[4], multipolar. In our study, we found them so poor in processes that they look almost apolar, but in all probability, the majority of them are either unipolar or bipolar. Sometimes, tripolar

Fig. 1

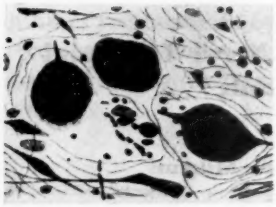

Fig. 2

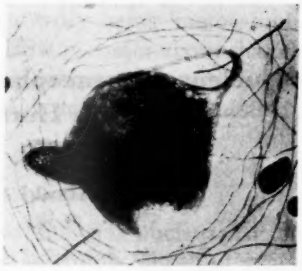

Fig. 3

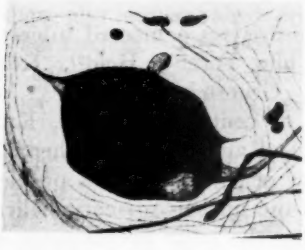

Fig. 4

Fig. 1. 3 sensory nerve cells and a number of vegetative nerve cells in nucleus mesencephalicus in a sagittal section of mesencephalon in man. Seto's silver impregnation. X 300, reduced to 2/3.

Fig. 2. A large sensory nerve cell and a vegetative nerve cell in the same section. Same statining. X 800, reduced to 2/3.

Fig. 3. Tripolar sensory nerve cell in the same section. Same staining. X 800, reduced to $\frac{1}{2}$.

Fig. 4. A wandering motor cell and nerve fibres connected with nucleus mesencephalicus in the same section. Same staining. X 300, reduced to 2/3.

cells as shown in Fig. 3 may be found, but in general, it is a characteristic of these cells that they are round in contour and have only extremely few processes.

These cells, accordingly, with their characteristic form, seem to be analogous in microscopic structure to the sensory cells found in the sensory nerve nuclei in the brain stem, especially in the medulla oblongata, and surely belong to the same category of nerve cells in nature, that is, to the sensory system.

In this nucleus, there are also found a smaller number of small-sized vegetative cells, similar to those found by Atsuki[16)17)] in the nuclei n. oculomotorii and n. trochlearis (Figs. 1 and 2). These cells are small in size, with a characteristic large cell nucleus. Kohnstamm[23)] has described

cell group of similar shape in this nucleus, which he named nucleus intratrigeminalis.

Besides, in this nucleus, in very rare cases, multipolar cells strongly resembling motor cells in the nucleus n. oculomotorii are found (Fig. 4). We presume that these are motor cells wandering into the area from the motor nuclei situated around the nucleus mesencephalicus, especially nucleus n. trochlearis. Formerly, Kölliker[3] detected some nerve cells with a number of processes in the nucleus, which might have been these errant cells we observed.

We must mention that no decision has been reached as to the nature of the nerve fibres connected with the nucleus mesencephalicus. E.g., Kure[21] Kosaka[13] have called them centrifugal, while May-Horseley[12], Probst and Lewandowsky[25] assert admixture of centripetal fibres originating in radix ascendentis n. trigemini, among them. Some would limit the course of the centrifugal fibres to the ganglion semilunare Gasseri, while others say they might be pursued up to the musculus masseter. Terterjanz[5] and Igarashi[9] define them as motor, from the results of their experimental researches.

Our view on the question is as follows. This nerve bundle, consisting of thick medullated fibres and probably unmedullated fine vegetative fibres, runs along the outer edge of this nucleus on the dorsal side of the crus cerebello-cerebrale in the vicinity of the decussation of vellum medullare anterius and radix n. trochlearis, showing a crescent form in horizontal section, which becomes thinner as the upper level is reached, and finally goes over into the fasciculus longitudinalis medialis. The fibres contained are apparently represented by sensory and vegetative fibres originating in the long processes of the nerve cells in this nucleus. At the caudal end, they reach the outside of the motor nucleus of trigeminal nerve in the vicinity of the nucleus loci caerulei, but according to Obersteiner[20], a part is said to penetrate into the sensory nucleus terminalis n. trigemini. This bundle is also composed of thick medullated and thin unmedullated fibres, the former seemingly coming from the ganglion Gasseri into the nucleus mesencephalicus, and the latter being represented by large processes of vegetative nerve cells in this nucleus or other external vegetative fibres entering into the nucleus.

Summary

The nucleus mesencephalicus n. trigemini in man consists of many roundish sensory nerve cells with very few processes and a small number of small-sized vegetative nerve cells, in very rare cases containing wandering motor cells presumably from the nucleus n. trochlearis.

The nerve bundle connected with this nucleus is composed of thick medullated fibres and thin unmedullated fibres. The superior fibres, which finally merge with the fasciculus longitudinalis medialis, seem to be represented by the long processes of the nerve cells in this nucleus, i.e. sensory and vegetative fibres, while the inferior fibres, which finally attain the outside of the motor nucleus n. trigemini, apparently consist of sensory fibres coming into the nucleus mesencephalicus from the ganglion Gasseri, and vegetative nerve fibres either from the vegetative cells in this nucleus or other external vegetative fibres running into the nucleus.

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Spinal Ganglia in Human Embryo, especially, in its Earlier Stage

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Histological studies on the cerebrospinal ganglia of adult man and mammals have been conducted by Dogiel,[1] Lenhossék,[2] Retzius,[3] Cajal,[4] Ranson,[5] Daae,[6] Kölliker,[7] and many other investigators, and more recently, by Stöhr,[8] Yamashita[9] and Seto,[10] which led to many remarkable results. On the other hand, such studies with embryonic specimens have been very few if anything and little has been elucidated on the problem viewed genetically. In particular, almost no research has been reported with human embryo as objective.

With a view to throwing light upon this problem, I took my materials from human embryos of earlier months (1st, 3rd and 4th month), and also from a 2nd year infant, for the purpose of tracing the development from embryonic to adult stage, fixed them in 10% neutral formol, cut into 40μ transverse frozen sections and stained with Seto's silver impregnation in use at this laboratory. The series of the numerous very beautiful preparations I subjected to minute microscopic study, and upon collation of my researches with the genetic observations by the predecessors and the histological figures in human adults, arrived at many interesting observations, which I will detail hereunder.

Individual Observations

It has been recognized from earlier times that the development of the nerve system is nearly completed much earlier than that of the other tissues and organs, and this proposition has been apparently emphatically endorsed in my embryonic studies with my silver impregnated preparations. For example, the development of the peripheral nerve system is very remarkable in the earlier embryonic stages, and what interested me most was that the nerve cells in the cerebrospinal ganglia are larger in size than any nerve cells in the central nerve system, that the ganglia themselves are developed enormously larger in comparison with the other

Fig. 1

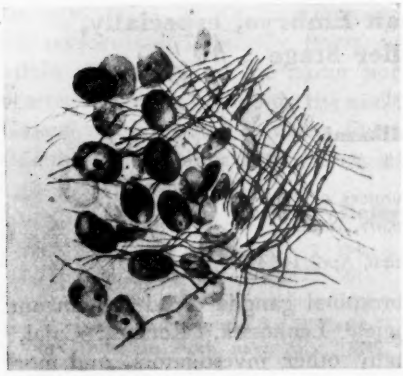

Fig. 2

Fig. 1. Large nerve cells with large spherical cell nucleus and almost untraceable incipient part of nerve processes, found in the vicinity of the distal pol of cervical ganglion and strongly stained nerve fibres running from the ganglion to the periphery. 1st month human embryo. Oblong cell nuclei are Schwann's nuclei. Cross section. Seto's impregnation. X 1100, reduced to 3/5.

Fig. 2. Cervical ganglion (s), nerve bundles entering and leaving it and sympathetic ganglion (sy). 3rd month human embryo. d radix dorsalis; v radix ventralis; rd ramus dorsalis; rv ramus ventralis; t truncus symp. Cross section. Same staining. X 40, reduced to $\frac{1}{2}$.

organs, and that the processes from their nerve cells and the motor nerve fibres originating in cornu ventrale of the spinal cord are both very strong in their affinity to silver, staining dark violet observed as very stout fibres (Figs. 1, 2, 3 and 4), as well as the fact that these fibres are observable to their terminations in their periphery. But as the terminal formations of these fibres are very simple, the later development is apparently accomplished gradually in the latter embryonic life and after birth.

The development of the peripheral vegetative nerve system is also very remarkable, nerve fibres being represented by minute fibres, clearly distinguished histologically from the thicker spinal fibres, with terminal formation represented by Stöhr's terminalreticulum, especially in the 3rd and 4th month embryos. It is noteworthy that the development of the nerve system, especially the peripheral nerve system, is much in advance of the other organs.

The sensory nerve cells in the cerebrospinal ganglia in human adult can be classified into the larger and smaller in size, as has been clarified

Fig. 3

Fig. 4

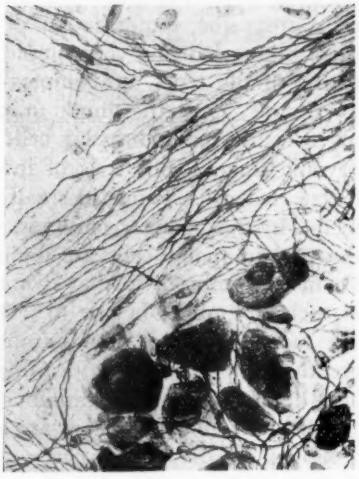

Fig. 3. Magnification of the distal pole of the above ganglion. Nerve cells are mainly of large type, the running course of the nerve fibres is remarkably distinct. Same staining. X 200, reduced to $\frac{1}{2}$.

Fig. 4. Magnification of A of the above ganglion. Fine structure of the large cells is very clear, most of them are simple unipolar cells. Mantle-cell nuclei are represented chiefly as small oval nuclei. Same staining. X 1100, reduced to $\frac{1}{2}$.

by many investigators, among whom Yamashita[9] must be mentioned as most dependable. From the number and various courses of their nerve processes, they can be again classified into the simple unipolar, the complex unipolar, the simple bipolar, the complex bipolar, the multipolar, the fenestrated, and the cells with end plates. I observed a situation similar to that observed by Yamashita also in my 2nd year infant, but the differentiation of the nerve cells was much weaker.

From the above, it may be inferred that the nerve cells in the cerebrospinal ganglia in the earlier embryonic life are yet more simple in formation than those in the infantile. In fact, in the cerebrospinal ganglia in the 3rd and 4th month embryos, to speak nothing of the 1st month embryo, the processes from the nerve cells never run complex courses, but are limited to very simple running courses of infantile type. But I found that the cells can be classified into the smaller and larger types, already in the 1st month embryo, the former chiefly being distributed at the proximal pole of the ganglia, especially at the circumference thereof, and the latter located in the vicinity of their distal pole.

The nerve cells in the cerebrospinal ganglia in the younger embryos

are in general distinguishable into the unipolar, bipolar and pseudo-apolar cells, from the conformation of their nerve processes. The courses of these processes being very simple as mentioned above, all the cells belong to the simple types, no complex-typed cells as seen in adult being found anywhere. Only the simple unipolar, the simple bipolar and the simplest my so-called pseudo-apolar cells can be distinguished in the earlier embryonic stages. But it is of interest to note that some multipolar and fenestrated cells, found in small number in adult, can be not rarely detected in the younger embryos, too. Of the above three types of cells, the pseudo-apolar cells are found in an especially large quantity in the 1st month embryo, so that it may be said that almost all the nerve cells in such an embryo belong to this type of cells. However, in the 3rd and 4th month embryos, the number of the pseudo-apolar cells diminishes greatly, unipolar cells appearing in their lieu, existence of bipolar cells also, though in a small number, being proven.

The above observations are at great variance with the theory hitherto accepted on the young nerve cells in the cerebrospinal ganglia of the embryonic life, which is a fact of some interest, but the details on this question will be taken up later on, and I will proceed with a brief discussion on the mantle-cell plasmodium (Stöhr's Nebenzellenplasmodium) and the connective tissue capsule around the nerve cells.

As may be inferred from the simple running of the processes from the nerve cells in younger embryos, the development of the mantle-cell plasmodium and connective tissue capsule around the nerve cells are as yet very imperfect. In the 1st month embryo, few connective tissue cells are observable in the ganglia, and as the number of mantle-cells corresponding to the glial cells is very small, the ganglia may be described as entirely taken up by nerve cells (Fig. 1). As the mantle-cells in the 1st month embryo are represented by small elongated cells much smaller than the nerve cells, the histological distinction between the two is very easy.

In the 3rd month embryo, the nerve cells get processes with conspicuous incipient part showing somewhat winding course, as described below. Inferring from the complex development of the incipient part of the processes in adult, an increase of mantle cells around the nerve cells, especially around the incipient part of the processes, is presumable, abreast with the development of the incipient part of the processes, and in fact, I found the gathering of mostly oval mantle-cell nuclei around the nerve cells, especially at the incipient part of the processes, wherever it is somewhat differentiated and developed to some extent (Fig. 4). The connective tissue cells are also a little more in evidence, but they do not as yet form pericellular connective tissue capsule, as in adult.

In 4th month embryo, the above described pericellular cell elements become a little more distinctly developed, especially the enlarged oval mantle-cell nuclei increasing remarkably, as shown in Figs. 4, 5 and 6. The development of the connective tissue around the cells is as yet very poor, so that no connective tissue capsules may be said to have formed.

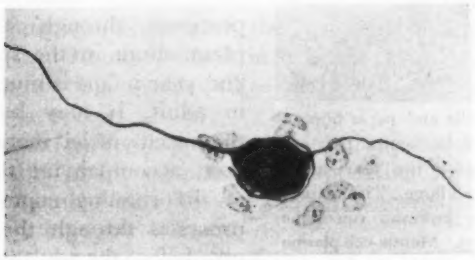

Fig. 5. A small multipolar cell found in a cervical ganglion. 3rd month embryo. Some oval mantle-cell nuclei are arranged irregularly around the nerve cell. Same staining. X 1100, reduced to 2/3.

Fig. 6. A small unipolar cell found in a cervical ganglion. 4th month human embryo. Mantle-cell nuclei are crowded near the incipient part of the nerve process. Both of the branched fibres are nearly equal in thickness. Same staining. X 1100, reduced to 2/3.

However, in 2nd year infant, the connective tissue capsule around the ganglia as well as the connective tissue elements within the ganglia are remarkably developed, the pericellular connective tissue capsule also reaching a stage of perfection. The mantle-cell plasmodium also becomes well developed, the cell nuclei in it appearing spherical in shape and arranged almost in a single layer around the nerve cells. Where the nerve processes show a complex course in the mantle-cell plasmodium, especially many mantle cell nuclei are found existing (Figs. 7 and 8).

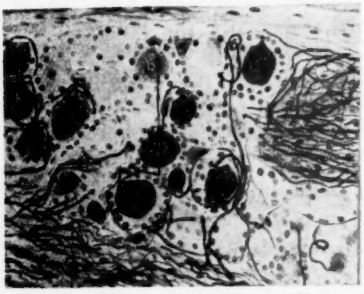

Fig. 7. Nerve cells and nerve fibres at the circumference of the central part of a thoracal ganglion of a two years infant. The nerve cells are all large. The incipient parts of the nerve processes run somewhat complex courses. Mantle-cell plasmodium and connective tissue capsule around the nerve cells are nearly perfected. Nerve fibres are composed of thick sensory and thin vegetative fibres. Same staining. X 320, reduced to 2/3.

The increase of mantle-cell nuclei, as cited above, is presumed to go abreast with the development of the nerve processes running through the mantle-cell plasmodium, that is, the gradually complicated running course. Now, as the running course of the processes through the mantle-cell plasmodium in the spinal ganglia of 2nd year infant is much simpler than in adult, it may be inferred that these cell nuclei increase much further, accompanying the complication of the running course of the nerve processes through the cell plasmodium, before the adult stage is reached.

The most infantile type of the nerve cells in the cerebrospinal ganglia in the earlier stages of embryonic life is presumably the my so-called pseudo-apolar cells. In 1st month embryo, almost all the nerve cells in the spinal ganglia are represented by this type (Fig. 1). The fact that this type of cells diminishes in quantity as the 3rd and 4th months are reached in the embryonic life betrays this type to be the most infantile.

The reason why I propose to call these cells "pseudo-apolar" is as follows. These cells have large-sized round cell nuclei, with protoplasm very poorly developed around them, so that the cells appear as if only composed of nuclei. Nerve processes from these cells are not easily observable, even under high magnification. However, as many thick sensory fibres, very conspicuously impregnated, are seen entering and leaving the spinal ganglia even in 1st month embryo (Fig. 1), it is evident that these nerve fibres originate in my so-called pseudo-apolar cells in the ganglia. So of course, the incipient part of these nerve processes must be formed, but as the neuro-fibrils therein, as around the cell nuclei, are in extremely infantile development, showing little affinity to silver as yet, it is presumed that the incipient part of the processes is not clearly observable.

A similar obscuration of the incipient part of the nerve processes while the peripheral part is clearly observed is found also by the motor nerve cells in cornu ventrale of the spinal cord in 1st month embryo—one of the most interesting phenomena coming under my observation. The stout fibres originating in the pseudo-apolar cells partly run far away

to the periphery of the body to end freely, partly run centrally into the cornu dorsale of the spinal cord through the radix dorsalis. The development of these fibres is already very marked in 1st month embryo.

I presume these pseudo-apolar cells grow into the undermentioned indubitable unipolar, bipolar and multipolar cells, as the neuro-fibrils at the incipient part of the processes and around the cell nuclei are matured and come to get silver affinity. In short, my so-called pseudo-apolar cells may be interpreted as representing the cells of most infantile development in the spinal ganglia. They have the incipient part of the processes formed in fact, but the neuro-fibrils in it being immature, are not made visible by silver staning, so that these nerve cells appear as lacking in nerve processes or apolar.

In 3rd month embryo, the number of the pseudo-apolar cells decreases remarkably, many unipolar cells appearing in their place. In a much smaller number, bipolar cells also are observed, and more rarely, fenestrated and multipolar cells.

According to my above observations, the hitherto accepted theory on the genetics of the sensory nerve cells in the cerebrospinal ganglia, which premises that the infantile type of such nerve cells is represented by a bipolar cell type, whereof the two processes gradually come together and fuse into one process to become a unipolar cell (van Gehuchten,[11] Lenhossék[2] and Cajal),[4] must be looked upon as radically exploded. This fact can also be deduced from the structure of the young nerve cells in the spinal ganglia detailed hereunder. Yamashita[9] has discovered a considerable quantity of bipolar cells, which were believed to disappear entirely after birth, in the Gasserian Ganglion in adult, and concluded that the bipolar cells do not represent infantile cells, but constitute an ordinary type of cells in the cerebrospinal ganglia, and this assertion also puts the past genetic hypothesis in doubt. I of course concur with the proposition of Yamashita from my genetic point of view.

The polar cells in the spinal ganglia of 3rd month embryo comprise extremely minute neuro-fibrils clearly silver staining and arranged reticularly around the cell nuclei. The incipient parts of the nerve processes also come to be filled with silver staining neuro-fibrils, going over into the peripheral strongly staining nerve processes.

Unipolar cells (Figs. 6, 8 and 9) are found in 4th month embryo more than in 3rd month specimen. They have one each process succeeding a not so stout incipient part of conical shape. In the incipient part, there are generally somewhat more of mantle-cell nuclei than around the nerve cell. The nerve processes go out of the plasmodium after running a simple slightly winding course respectively. Typical cells of this unipolar type are found mainly in the large nerve cells seen near the distal pole of the

Fig. 8

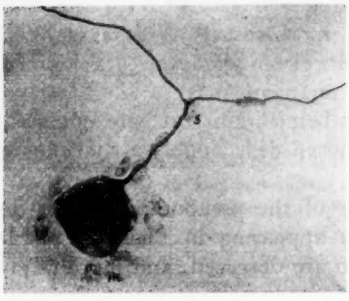

Fig. 9

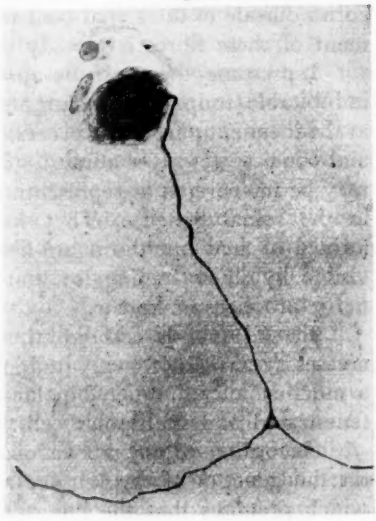

Fig. 8. A large unipolar cell found in a cervical ganglion. 3rd month human embryo. m Mantle-cell nuclei; s Schwann's nuclei; both branch fibres show unequal in thickness. Same staining. X 1100, reduced to 2/3.

Fig. 9. A typical large-sized unipolar cell found in a cervical ganglion. 4th month human embryo. Nerve process divides into two branches after running a considerable distance. The branching point shows a triangular neuro-fibrilar dissolution. Both the branch fibres are approximately same in size. Same staining. X 1100, reduced to 2/3.

ganglia, as stated above (Figs. 3 and 4). The processes from these cells run from the circumference of the ganglia toward the center, generally convergingly, and soon bifurcate in T- or Y-form, but generally not before they have fused with the nerve fibre bundles at the centre. Thus, it has been established that the bifurcation of nerve processes is accomplished at a considerable distance from the mother cells, in the embryonic, especially in the early embryonic stage. This fact also contradicts the hitherto accepted hypothesis that the infantile type of the sensory nerve cells in the cerebrospinal ganglia is represented by bipolar cells. In adult, the nerve processes bifurcate in many cases not far from the mother cells. This phenomenon is attributable to the fact that the incipient parts of the processes run complicated courses in the mantle-cell plasmodium by mature cells, so that the processes are drawn nearer the mother cells.

The nerve processes of the unipolar cells as well as the bipolar and multipolar cells are at their incipient part sometimes represented by stout fibres showing change or increase in size due to neuro-fibril dissolution

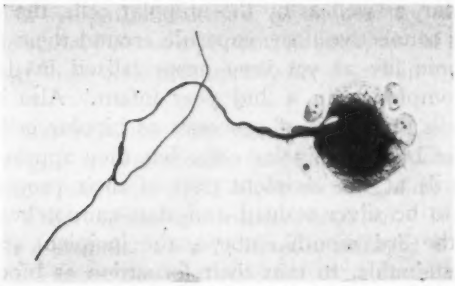

Fig. 10. A large unipolar cell found in the same. Incipient part of the process is represented by a thick fibre due to neuro-fibril dissolution. Same staining. X 1100, reduced to 2/3.

(Fig. 10), but in the more peripheral part by smooth, strongly silver staining fibres of nearly unchanging size. The bifurcated point of the nerve processes very often forms a triangular neuro-fibril dissolution, as shown in Fig. 9. The peripheral and central fibres branching out thus do not show any conformation to a rule in size, like in the Yamashita's observations by the ganglion Gasseri in adult. In some cases, the two branches are nearly equal, but in other cases far from equal, the size of the branches often being not at all smaller than the stem fibre. The theory that the bifurcated branches are smaller than the stem fibre, the total sum of the diameters of the two branches being equal to the stem, in the nerve cells of the spinal ganglia in frog, proposed by Lenhossék, and the assertion by a few researchers that the peripheral branches are thicker than the central branches, have not found general applicability in my study of the spinal ganglia in human embryos, and neither in that of a 2nd year infant.

As stated above, the bipolar cells, according to the theory accepted hitherto, are infantile cells representing the nerve cells in the cerebrospinal ganglia in the embryonic stage, to be soon developed into unipolar cells, so it was held that if bipolar cells are found there after birth, these represent remnants of indifferentiated lower state. However, Yamashita[9] observed existence of not a few bipolar cells in adult specimens, and proposed that these are not infantile remnants but represent a normal cell type. In my study on embryos, I have arrived at nearly the same conclusion as Yamashita concerning the bipolar cells, which fact endorses the accuracy of Yamashita's proposal. As bipolar cells are found in much smaller number than the unipolar cells even at the earlier stage of embryonic life, it is clear that bipolar cells do not appear as a infantile cell type, but bipolar cells develop as bipolar throughout and unipolar as unipolar.

By the bipolar, as well as by the unipolar cells, the mantle-cell plasmodium and the connective tissue capasule around them are in the earlier stage of embryonic life as yet very unspecialized in development, but come to near completion in a 2nd year infant. Also by my so-called pseudo-apolar cells the germs of processes as bipolar cells are developed early enough, like by the unipolar cells, but they appear as apolar, only as the neuro-fibrils at the incipient part of their processes are not yet mature enough to be silver stained and thus cannot be detected microscopically. In the 3rd month embryo, the incipient parts are already markedly silver stainable, so that their formation as bipolar cells is here clearly established.

The distance between the starting points of the two processes from the bipolar cells in the earlier embryonic stages is rather variable, sometimes the two emerging from each opposite cell pole, but sometimes from two points rather very close indeed (Fig. 11), with many transitory

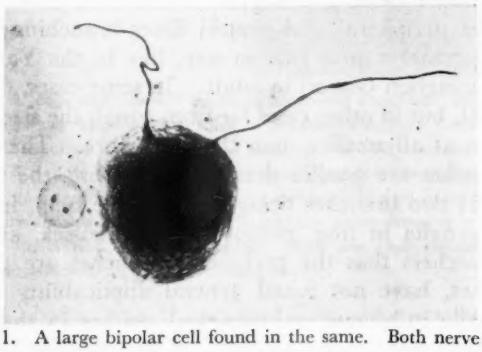

Fig. 11. A large bipolar cell found in the same. Both nerve processes are of nearly same size. Same staining. X 1100.

types lying between them. The incipient part is often represented by thick fibres of variable size, thickened by neuro-fibril dissolution, as is the case with unipolar cells. The running course of this part does not show any complexity, but sometimes a simple loop-like course is observed, and finally it runs through the mantle-cell plasmodium outwards. Then, the process becomes smooth-surfaced fibre almost without change in size. Between the two processes by the bipolar cells, as by the bifurcated branches of unipolar cells, there applies not rule governing their relative sizes.

It has been discovered by Dogiel,[1] Lenhossék,[2] de Castro,[12] Cajal,[4] Ranson,[5] Barris[13] and Yamashita[9] that the multipolar cells exist in the cerebrospinal ganglia in human adult, though in a very limited number. In particular, Yamashita clarified that these multipolar cells are mor-

phologically not of sympathetic nature, as claimed by some investigators, but belong to the sensory nerve cells, and as such, are of normal existence. He also states that these cells presumably are found in existence in infants already.

Sclavunos[14] has reported the existence of such multipolar cells in cerebrospinal ganglia in embryonic stage, but his observation was of a goat fetus studied with the old Golgi's method, and judging from his illustrations, his observations are rather doubtful. No report on the existence of such multipolar cells in human embryos is known.

In my study, I came to an observation that clearly endorsed Yamashita's assumption. In my 3rd month and 4th month embryos, I found some very distinctly formed multipolar cells, small in number, but clearly of the same type as found in human adult. Their short processes were even numerous, comparatively speaking, numbering 2–4 per cell, which often branched out or showed neurofibril dissolution, a state of some interest (Figs. 5, 12 and 13). As the existence of multipolar cells having morphologically normal short processes is found in the cerebrospinal ganglia of embryos in earlier stages, it goes without saying that the existence of a normal type of multipolar cells in human adult does not show any pathological state.

The so-called fenestrated cells assumed to be variation of the above multipolar cells, which are characteristic with the formation of nervous anastomosis between short nerve

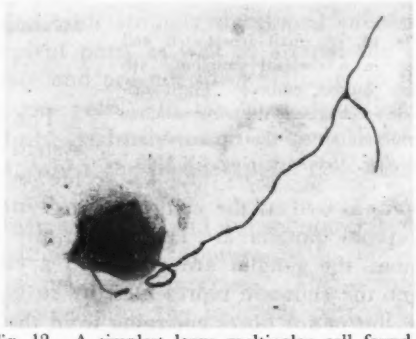

Fig. 12. A simplest large multipolar cell found in the same. Short process ends in a small terminal plate in the mantle-cell plasmodium. Long process, after describing a simple loop in the plasmodium, runs a rather long course before dividing into two branches nearly equal in thickness. Same staining. X 1100, reduced to 2/3.

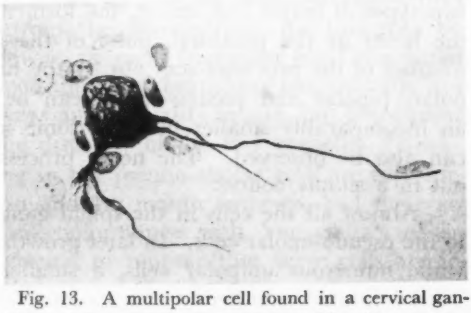

Fig. 13. A multipolar cell found in a cervical ganglion. 3rd month human embryo. The short process on the left side shows fibril dissolution and branching. Same staining. X 1100, reduced to 2/3.

Fig. 14. A small fenestrated cell found in a cervical ganglion. 4th month human embryo. Fibril dissolution seen around the nerve window, wherefrom a few short processes are emerging. Same staining. X 1100.

processes, are also found in the adult cerebrospinal ganglia. According to Yamashita, they do not show any pathological appearance, and must be looked upon as a normal cell type. In my study on embryos, I found existence of similarly constructed fenestrated cells (Fig. 14). Thus, it is clear that these fenestrated cells are formed already in the embryonic life, as are the multipolar cells, and I concur with Yamashita in assuming these to be normal cells.

Summary

The development of peripheral nerve system in the earlier stage of embrynic life is much in advance to the other organs as well as the central nerve system. The nerve cells in the cerebrospinal ganglia are larger than any nerve cells in the central nerve system, the ganglia are grown to a enormous dimension, the processes from the cells are represented by strongly silver staining thick fibres, as are the motor fibres emerging from the ventral horns of the spinal cord, and these fibres are clearly traceable to the farthest periphery. The development of the peripheral vegetative nerve system is also very remarkable, the fibres being of the minute type and the termination being formed into the distinct terminalreticulum (Stöhr) in the 3rd and 4th month embryos already.

The sensory nerve cells in the spinal ganglia are classifiable into the two types of major and minor, the former being found at the distal, and the latter at the proximal, poles of the ganglia in general. From the number of the processes sent out by the unit cells, the three types of unipolar, bipolar and pseudo-apolar can be distinguished. But though in an incomparably smaller number, some multipolar and fenestrated cells can also be observed. The nerve processes from these cells always run out in a simple course.

Almost all the cells in the spinal ganglia of 1st month embryo belong to the pseudo-apolar cells. In later growth, these cells decrease in number, while numerous unipolar cells, a smaller number of bipolar cells and a very limited number of multipolar cells make appearance. This observation is widely at variance with the hitherto accepted genetic theory about these nerve cells.

The mantle-cell plasmodium and the connective tissue capasule

surrounging the nerve cells are very poorly developed in 1st month embryo, but in 3rd and 4th month embryos, mantle-cells are observed in an increased number around the nerve cells, especially at the incipient part of the nerve processes, simultaneously with the conspicuous appearance of the incipient part, and also the development of the connective tissue capsules shows some progress. The development of these cell elements around the nerve cells reaches a nearly final stage in 2nd year infant. Thus it is presumed that the increase of the mantle-cell nuclei keeps abreast with the complication of the nerve processes running through the mantle-cell plasmodium.

The pseudo-apolar cells represent the most infantile cell type in the spinal ganglia. They are provided with strongly developed processes, but the nerve fibrils at their incipient parts as well as around the cell nuclei are yet in an immature state and are not silver staining, so that these cells appear as if they lack any poles. In the later stages in the embryonic life, the immature fibrils gradually come to maturity and their silver affinity grows with it, when the pseudo-apolar cells assume the shape of polar cells.

Polar cells are very clearly observed in 3rd and 4th month embryos. Minute neuro-fibrils with silver affinity make appearance in the cell bodies and the incipient parts of the nerve processes. The unipolar cells have one process each continuous from the incipient part, which runs through the plasmodium in a very simple winding course, and the process branches out in T- or Y-shape at a considerable distance from the mother cell. However, as the running course of the incipient part grows more complicated with the growth of the body, and the process is attracted to the mother cell, the branching point apparently comes also nearer to the mother cell in adult.

The incipient part of the processes, as in other cells, often show fibril dissolution. Upon emergence from the plasmodium, the processes become nearly constant in size, showing frequent triangular fibril dissolution at the branching points. Between the fibres branching out centrally and peripherally, there is almost always an unequality in size, but there seems to be no regularity govering the disparity of the two branches.

The bipolar cells originating in the pseudo-apolar cells are very conspicuously represented in the 3rd and 4th month embryos, and these are permanent and normal cells, in conformance with Yamashita's observation in adult. There is no ground to suppose that these cells are infantile type of cells in cerebrospinal ganglia, as some past researchers have assumed. The two nerve processes originating from such a cell appear at various mutual distances as by the adult specimens (Yamashita). At the incipient part of the processes there appear neuro-fibril

dissolutions as by unipolar cells, the running courses of the processes are very simple, and between the two processes running out of the mantle-cell plasmodium, there is generally the same disparity of size as prevails between the bifurcated branches by the unipolar cells.

The multipolar cells found in rare cases are of normal formation, as in adult, and do not belong to the sympathetic system. The short processes from these cells sometimes show branching and fibril dissolution. The fenestrated cells, a variation of multipolar cells, formed by the anastomosis between the short nerve processes, are rarely found in the spinal ganglia of embryos in earlier stages, and these also are normal cells, which, according with what Yamashita has observed in his adult specimens, are destined to last from the early embryonic stage to the adult maturity.

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Comparison of the Group Carbohydrate of Gastric Cancer with the Corresponding Carbohydrate of Gastric Mucosa*

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Investigations on the group carbohydrates (amino acid-polysaccharide complexes) from gastric cancer and gastric mucosa were reported formerly from this Institute (Refer to Oh-Uti[1], Satoh[2] and Masamune *et al.*[3]). In continuation of those works a further study has been made on preparations of them, one from each batch of the tissues of Group A. Electrophoresis demonstrated that the preparation from the cancers was somewhat contaminated with the mucosa carbohydrate although the cancers had been freed from the intact neighbourhood most carefully. The results are summarized:— 1) The substances both proved to contain chondrosamine, glucosamine, galactose and L-fucose as sugar components and aspartic and glutamic acid, serine, glycine, alanine, threonine, histidine, arginine, proline, valine and/or methionine, leucine and/or isoleucine as amino acid components. 2) They also resembled each other in quantitative analyses excepting iodine value. The iodine value of the carbohydrate from the mucosa amounted to nearly twice the value of the one from the cancers, which is probably ascribed to its alkaline cleavage during preparation (Cf. below). 3) The both substances reduced about 3.2×10^{-3} mole of HIO_4 per g., under disruption of chiefly non-amino sugars in their molecule, suggesting that, in both of the carbohydrates, 3- or more probably 4-C of amino sugars and 4- or more probably 2-C of non-amino sugars (Pyranose structure of all the hexose units are assumed) take part in O-glucosidic linkage between the adjacent hexose units. 4) The cancer carbohydrate was much less potent in isohemoagglutination

* The 160th report of Masamune and coworkers' "Biochemical Studies on Carbohydrates." The most part was read before the 24th General Meeting of the Japanese Biochemical Society, Kobe, May 3-5, 1952.[5]

inhibition than the mucosa carbohydrate. 5) The substances, however, showed no discernible difference of antilytic power. 6) They were deprived of the serological activities in 4) and 5) by periodic acid. 7) Regarding the partial antigens, the cancer carbohydrate had much less of A_I and A_{II} than the mucosa carbohydrate, whereas they nearly equaled each other in A_{III} and A_{IV} contents. 8) The cancer carbohydrate exhibited distinct cancer skin reaction (Matsubara). This potency was destroyed neither by periodic acid oxidation nor by tryptic digestion. The mucosa carbohydrate also possessed it though feeble.

Taken altogether, the two group carbohydrates appear to be intrinsically (not perfectly) identical in so far as chemical structure concerns, but differ in biological aspect. The peculiarity of the cancer substance in 7) offers an additional argument for our view already pronounced[1] that it is biologically immature, i.e., of low-differentiation. On the other hand, the residue in the molecule of the cancer substance that gives rise to the cancer reaction is hardly sought in the proper carbohydrate moiety because of the positive reaction of the substance even after HIO_4-oxidation.

Experiments and Comments

A. Preparation of the Group Carbohydrates and their Properties

Preparation

Crude materials were purified by the copper-alkali procedure.[2]

The cancer carbohydrate (**C-Ch**). Cr. Ch. which had been set aside by Masukawa[4] during the fractionation of gastric cancer of Group A persons was taken as a material. 1.7 g. of it were dissolved in 30 cc. of water by the aid of NaOH (The substance dissolved almost perfectly at pH 9.0). To the solution were added 20 cc. of 10% NaOH and 15 cc. of 12.5% $CuSO_4 \cdot 5H_2O$ under agitation. Centrifuged. The centrifugate was washed with 3% NaOH repeatedly until the washing remained scarcely colored, and then stirred up with 15 cc. of 10% HCl. The insoluble was centrifuged off and the supernatant was precipitated with 5 volumes of abs. alcohol, washed with alcohol and dried. 0.8 g. of a white powder was given. The copper-alkali treatment was carried out twice more, whereby the quantity of substance was reduced to 0.65 g. Next it was taken up in 20 cc. of slightly alkaline water (pH 8.0) and the clear solution was acidified to pH 2.0. Some precipitate appearing thereupon was centrifuged off. The supernatant was precipitated again with 5 volumes of abs. alcohol, washed with alcohol and dried in vacuo over H_2SO_4. It contained still a tiny insoluble part, from which it was freed by reprecipitation. The yield of a white powder given was 0.55 g. It gave an acidic transparent solution in water.

The mucosa carbohydrate (**M-Ch**). 1.2 g. of Masukawa's Cr. Ch. of gastric mucosa[4] were treated similar to above. In this case the contamination insoluble at pH 2.0 amounted much less and could be eliminated entirely by a single operation. The final product (a white powder) was 0.5 g. It dissolved in water giving an acidic transparent solution as the cancer carbohydrate.

Electrophoresis

A 1% solution of pH 7.8 of the preparations gave patterns in Fig. 1. Neither of the substances was found homogeneous, a large and a tiny boundary being demonstrated in the mucosa substance and a third but biggest boundary besides in the cancer substance. Judging from their mobilities, the two smaller boundaries of the cancer substance was attributable to the group carbohydrate, as a contamination, of the mucosa remnants in the cancer material.

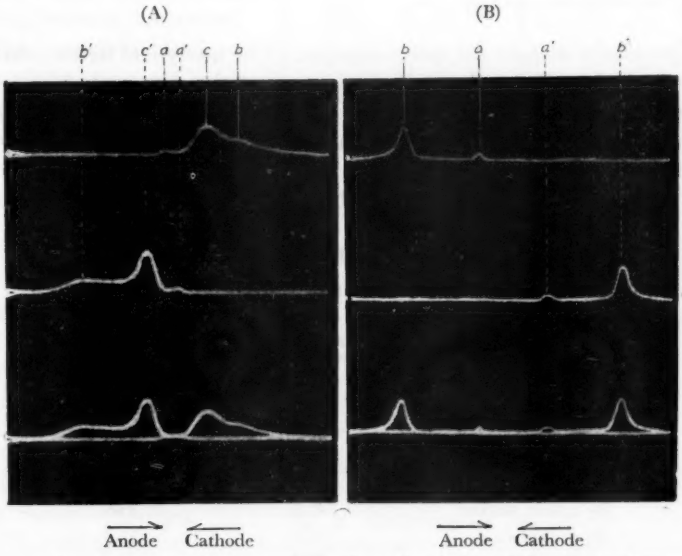


Fig. 1. Electrophoretic patterns of a 1% solution of the preparations from (A) the cancers and (B) mucosa in phosphate buffer, pH 7.8, I 0.29; 7°C; current 10 ma; exposure 60 minutes after starting current. Ascending limbs to the left. (A) and (B), different in gradient of the diagonal slit.

Mobility (A): a and a′, 29.4 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$
b and b′, 6.3 ,,
c and c′, 18.9 ,,
(B): a and a′, 29.2 ,,
b and b′, 6.3 ,,

Chemical properties

Qualitative assays. The results:

Assay	C-Ch	M-Ch
Biuret	−	−
Ninhydrin	−	−
Sakaguchi	+	+
Hiyama	+	±
Molisch	##	##
Reaction with Ehrlich reagent*		
After alkaline treatment	+	++
Without prior treatm.	+	−
Neuberg-Saneyoshi	−	−
Schiff (for aldehyde)	−	−
Hydrolysable sulfur	±	±
Phosphorus	−	−

−: negative, ±: faintly positive, +: slightly positive, ++: markedly positive, ##: strongly positive. * red coloration; spectral observation was not made carelessly.

Quantitative analyses and optical rotations. The analytical figures obtained are embodied in Table I which indicate that the two carbohydrates resembled each other in composition. The iodine values were at so much variance that the minimum molecular weight of the cancer carbohydrate approached twice the corresponding weight of the mucosa carbohydrate. However, purification of them had been effected by precipitation of the

TABLE I

Analyses and Optical Rotation of the Group Carbohydrates from Gastric Cancers and Gastric Mucosa

Figures express percentages excepting the iodine values and rotations.

Analysis	C-Ch	M-Ch
N*	5.0	4.3
Hexosamines†	39.1	40.9
Non-amino sugars as galactose		
By indole method	42.3	43.6
By orcinol m.	35.7	38.8
Average	39.0	41.2
Acetyl‡	11.8	9.9
Ash§	0.9	0.3
Iodine value//	3.6	6.9
$[\alpha]_D^{\gamma}$ in 0.01 *N* NaOH	−31.8°	−26.4°

* Micro Kjeldahl. † Hamasato & Akakura method. ‡ Suzuki's modification of Friedrich, Rapoport & Sternberg method. § Pregl method without use of H_2SO_4. // Macleod and Robison method.

copper complex salts at strong alkalinity which most probably cleft some alkali-labile link in the mucosa carbohydrate. As a matter of fact, in a trial avoiding such a drastic operation, preparations with iodine values 3.7 and 4.4 were obtained from the cancers and mucosa respectively, and the writers incline to believe the native substances similar or at most not to diverge much in molecular weight. The relationship between the optical rotatory forces was in agreement with the claim of Oh-Uti[1] that the Group A cancer carbohydrate is more levorotatory than the corresponding mucosa substance.

Paper partition chromatography of the sugar and amino acid components. 1) Sugars. The descending method of Masamune and Yosizawa[6] was adapted, empolying strips (5 cm. × 60 cm.) of Toyo filter paper, No. 2. 30 mg. each of the carbohydrates were hydrolyzed with 3 cc. of 1 *N* H_2SO_4 in an ampoule by heating at 100°C. The hydrolysates, nearly neutralized (to pH 5.8) with baryta, were centrifuged and the supernatant were distilled in vacuo to about 0.8 cc. and readjusted to pH 5.8 with baryta. Of the centrifuged supernatant here a 0.03 cc. portion each were sampled. As Figs. 2A and 2B demonstrate, the group carbohydrates both contained chondrosamine, glucosamine,

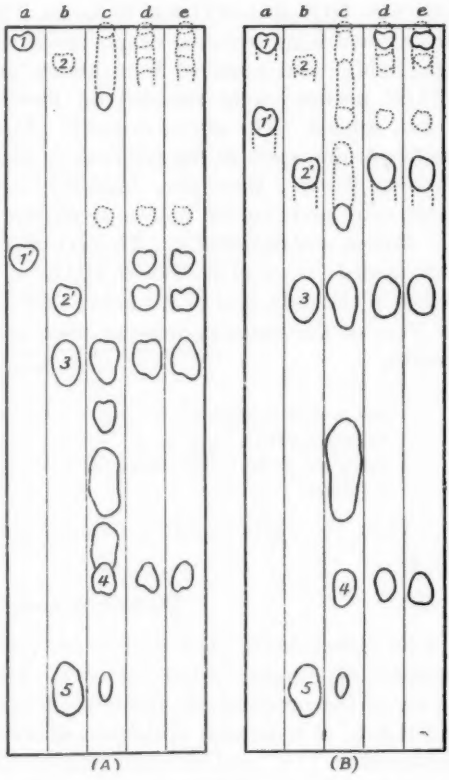


Fig. 2. Chromatograms irrigated with (A) *n*-butanol-pyridine-water (5:3:2) and with (B) *n*-butyl-acetate-acetic acid-ethanol-water (3:2:1:1) of the hydrolysate of C-Ch and M-Ch. Indicator: aniline hydrogen phthalate of Partridge. Temperature of test 22–26°C, duration of test 24 hrs.

a, b, c: reference runs (c, the chromatogram of a hydrolysate of traganth), d: cancer carbohydrate, e: mucosa carbohydrate. 1, 1′: chondrosamine sulfate, 2, 2′: glucosamine sulfate, 3: galactose, 4: L-fucose, 5: rhamnose.

galactose and L-fucose, but no mannose.

It must be very queer that the preparations[3] previously obtained in a manner similar to here contained mannose, which is difficultly explained at present.

2) Amino acids. 50 mg. each of the preparations were refluxed with 6 *N* HCl at 100°C for 30 hours. Then, HCl was expelled off as far as possible by repeated evaporations with water and a solution of the final residue was removed from humin and distilled in vacuo to 0.5 cc. 0.04 cc. portions were sampled on sheets of Toyo filter paper (No. 2) 25 cm. square. The chromatograms (Figs. 3–5) showed, in both of the carbohydrates, spots at the positions of aspartic and glutamic acid, serine, glycine, alanine, threonine, histidine, arginine, proline, valine and/or methionine and leucine and/or isoleucine.

Periodic acid consumption. To 5 cc. of a 3% solution of the preparations were added 10 cc. of 0.2634 *M* HIO_4, to follow Yosizawa[8] for determination of the reduction of the acid at 20°C. The titration was conducted on 3 cc. of the reaction mixture after cessation of the rotatory change. Results.

	C-Ch	M-Ch
Duration of rotatory change (hrs.)	3.0	3.5
Unreduced HIO_4 (moles)	0.4273×10^{-3}	0.4324×10^{-3}
HIO_4 use of the carbohydrates per g. (moles)	3.32×10^{-3}	3.15×10^{-3}

Thus the carbohydrates reduced nearly equal amounts of HIO_4 per g.

Biological properties

Serological assays (The writers are indebted to Dr. T. Miki in the Institute of Forensic Medicine). 1) Isohemoagglutination inhibition. 0.2 cc. of the progressively diluted solutions of the carbohydrates in saline and 0.2 cc. of a normal antiserum diluted to a titre of 8 were mixed and stood at 37°C and at room temperature for 1 hour each and at 0°C overnight in turn. Then 1 drop of the mixtures and 1 drop of 1% A or B erythrocytes were put together on a hollow objectglass to examine the agglutination after 30–45 minutes of standing at room temperature. The results are shown in Table II.

2) Antigen-structure of the carbohydrates. Agglutination of human A erythrocytes was examined similar to above by replacing the normal antisera with anti-partial antigen immune sera following.

a) Anti-A_I serum. A rabbit of Group O′ was immunized with human A erythrocytes. The serum obtained had titre 1600 towards human A erythrocytes, titre 10 towards human O erythrocytes and a

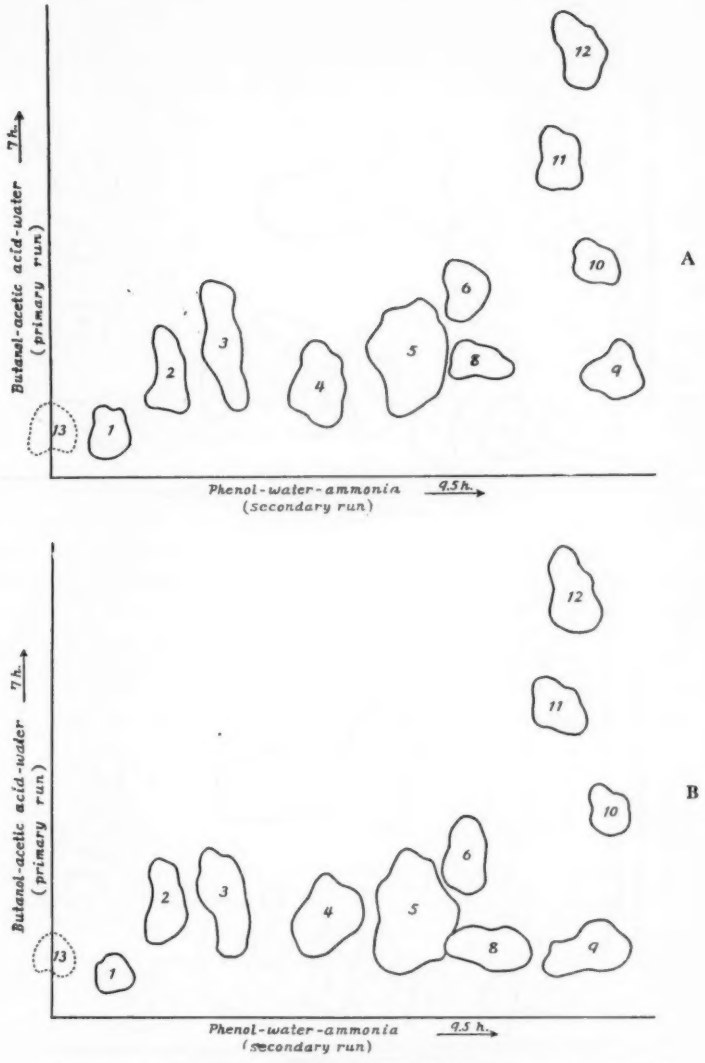


Fig. 3. *n*-Butanol-acetic acid-water (4:1:5)[7]/phenol-water (4:1)-ammonia chromatograms of a hydrolysate of (A) C-Ch and (B) M-Ch, developed with ninhydrin. Temperature of test 25–28°C.

1: aspartic acid, 2: glutamic acid, 3: serine, 4: glycine, 5: hexosamines, 6: alanine, 7: threonine, 8: histidine, 9: arginine, 10: proline, 11: valine and/or methionine, 12: leucine and/or isoleucine, 13: a peptide (?). (Some of these spots are not seen here.)

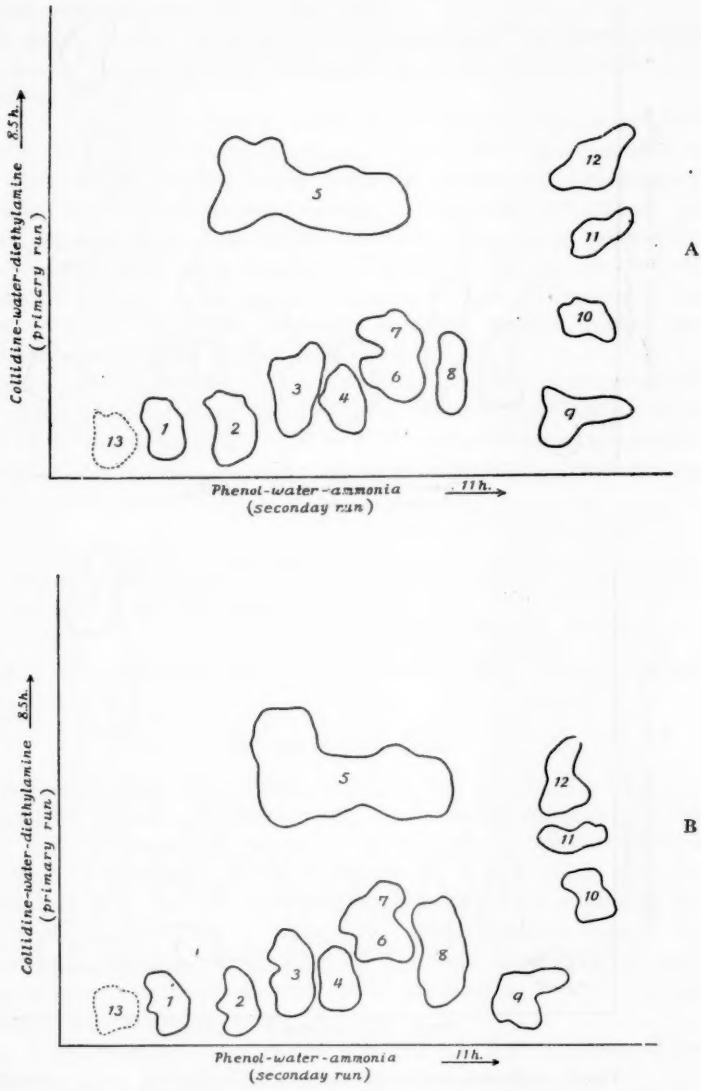


Fig. 4. Collidine (water-saturated)-diethylamine/phenol-water (4:1)-ammonia chromatograms of the hydrolysate of (A) C-Ch and (B) M-Ch, which gave the chromatograms in Fig. 3, developed with ninhydrin. Temperature of test 25–28°C.

Ciphers express the same as in Fig. 3.

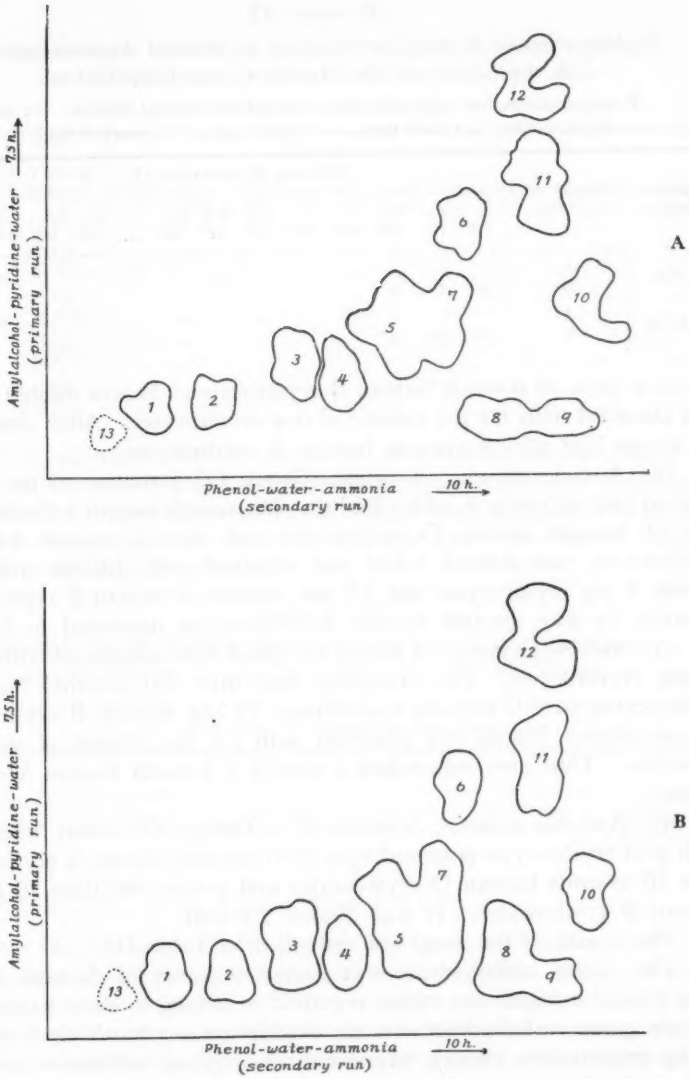


Fig. 5. Amylalcohol-pyridine-water (7:7:6)/phenol-water (4:1)-ammonia chromatograms of the hydrolysate of (A) C-Ch and (B) M-Ch, which gave the chromatograms in Fig. 3, developed with ninhydrin. Temperature of test 25–28°C.

Ciphers express the same as in Fig. 3.

TABLE II

Agglutination of Human Erythrocytes by Normal Antisera treated with the Cancer or the Mucosa Group Carbohydrate

B and A serum had agglutinin titre 160 and were diluted 20-fold. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl.

Carbohydrate	Group of red cells	Dilution of substances (1:)												
		10^2	2×10^2	4×10^2	10^3	10^4	2×10^4	4×10^4	8×10^4	...	8×10^5	1.6×10^6	3.2×10^6	without subst.
C-Ch	A				−	−	−	±	+	+	+	+	++	++
	B	++	++	++										++
M-Ch	A				−	−	−	−	−	−	−	+	+	++
	B	++	++	++										++

titre less than 10 towards human B erythrocytes. It was diluted 50-fold and absorbed with 1/4 the volume of dog erythrocytes. After absorption, the serum had titre 8 towards human A erythrocytes.

b) Anti-A_{II} serum. A rabbit (Group O′) immune serum against dog red cells of Group A_2 which had titre 80 towards human A erythrocytes, titre 40 towards human O erythrocytes and titre 20 towards human B erythrocytes, was diluted 4-fold and absorbed with 1/6 the volume of Group A pig erythrocytes and 1/6 the volume of human B erythrocytes, whereby its titre towards human A erythrocytes decreased to 8.

c) Anti-A_{III} serum. A Group O′ rabbit was immunized with Group A pig erythrocytes. The antiserum had titre 320 towards human A erythrocytes, titre 0 towards both human O and human B erythrocytes. It was diluted 10-fold and absorbed with 1/6 the volume of goat erythrocytes. Thus aborbed, it had a titre of 8 towards human A erythrocytes.

d) Anti-A_{IV} serum. A serum of a Group O′ rabbit immunized with goat erythrocytes possessed titre 1600 towards human A erythrocytes, titre 10 towards human O erythrocytes and a titre less than 10 towards human B erythrocytes. It was diluted 200-fold.

The results of the assay are embodied in Table III.

The cancer carbohydrate was found so scanty of A_I and A_{II} that these partial antigens are rather regarded to belong to the contaminating mucosa group carbohydrate (See the description as regards electrophoresis of the preparations above), whereas the two group substances compared with each other in the A_{III} and A_{IV} contents.

3) Hemolysis inhibition. 0.2 cc. of an anti-A rabbit (Group O′) serum, diluted to a titre of 4, and 0.2 cc. of one of the progressively diluted solutions of the carbohydrates were incubated together at 37°C and at room temperature for 1 hour each and in an ice chest (0°C) overnight,

TABLE III

Agglutination of Human A Erythrocytes by Antisera treated with the Cancer or Mucosa Group Carbohydrate

−: no aggl., ±: faint aggl., +: slight aggl., ++: marked aggl.

Antiserum of	Carbohydrate	Dilution of substances (1:)										
		3.2×10^{3}	6.4×10^{3}	1.28×10^{4}	2.56×10^{4}	5.12×10^{4}	1.024×10^{5}	2.048×10^{5}	4.096×10^{5}	8.192×10^{5}	1.6384×10^{6}	without substance
A_I	C-Ch	−	±	+	+	+	+	+	++	++	++	++
	M-Ch	−	−	−	−	−	±	+	+	+	++	++
A_{II}	C-Ch	−	−	±	+	+	+	+	++	++	++	++
	M-Ch	−	−	−	−	−	±	+	+	+	++	++
A_{III}	C-Ch	−	−	−	−	−	−	±	+	+	+	++
	M-Ch	−	−	−	−	−	−	−	−	+	+	++
A_{IV}	C-Ch	−	−	−	−	−	−	±	+	+	+	++
	M-Ch	−	−	−	−	−	−	−	±	+	+	++

followed by adding in sequence of 0.2 cc. of a 10-fold diluted guinea pig serum and 0.2 cc. of 2.5% sheep erythrocytes. Then the suspensions were placed in a water-thermostat of 37°C for 1 hour to examine hemolysis. Table IV illustrates that the two carbohydrates were almost equally matched in hemolysis inhibition.

TABLE IV

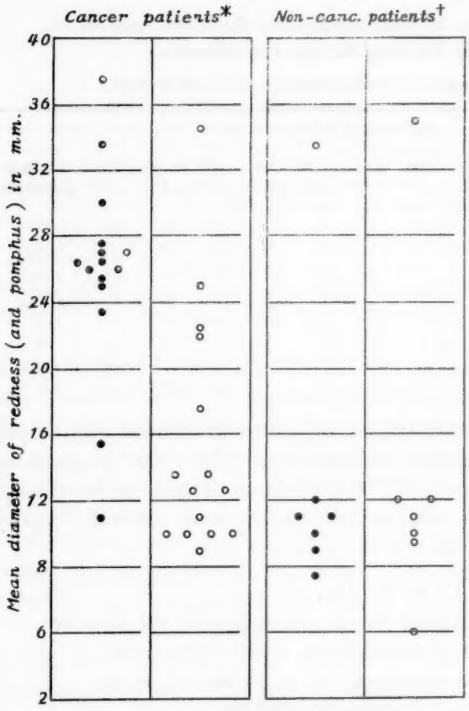
Hemolysis by the Anti-A Rabbit Serum, treated with the Cancer or the Mucosa Group Carbohydrate, and Complement

−: no hemolysis, ±: faint hemolysis, +, ++, ++ and ++: partial hemolysis, ++: complete hemolysis.

Carbohydrate	Dilution of substances (1:)										
	10^{4}	5×10^{4}	10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	1.6×10^{6}	3.2×10^{6}	6.4×10^{6}	without complement	without complement and substance
C-Ch	−	−	±	+	++	++	++	++	++	−	−
M-Ch	−	−	−	±	++	++	++	++	++	−	

Cancer skin reaction. The technique of Masukawa[4] was applied with a 0.1% solution of the carbohydrates in physiological saline. Results are diagrammatized in Fig. 6. The cancer carbohydrate was found evidently capable of giving the positive reaction. The mucosa carbohydrate also had the tendency though not so distinct. Under the speculation that the peculiar structure for the reaction may constitute a part of the peptide chain, the effect of trypsin on the potency was examined: To 10 mg. of the cancer carbohydrate in 1 cc. of water were added 10 mg. of trypsin (Kahlbaum) and the pH of the solution was brought to 9.0 with 1% Na_2CO_3. It was incubated (Toluene was used as a preservative)

Fig. 6



F g. 7

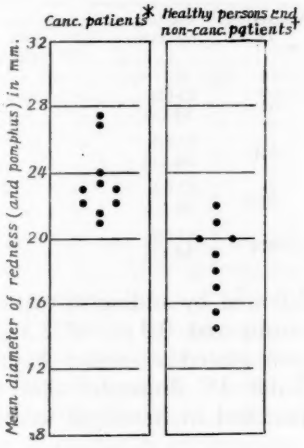


Fig. 6. Showing the skin reactions of C-Ch and M-Ch (0.1% solutions).
• the cancer substance. ∘ the mucosa substance. * carcinoma of stomach (7), pancreas (3), liver (2), rectum (1), oesophagus (1) and bile ducts (1). † gastric ulcer (2), chronic cystitis (1), internal haemorrhoids (1), pleuritis (1), renal tuberculosis (1) and fracture (1).

Judgement: the cancer substance, positive; the mucosa substance, positive.

Fig. 7. Showing the skin reactions of C-Ch digested with trypsin (a 0.1% solution).

* carcinoma of stomach (4) and rectum (4), kidney tumor (1), and lymphosarcoma (1). † healthy persons (3), gastric ulcer (1), appendicitis (1), colitis (1), hernia (1), haemorroids (1) and fracture (1).

Judgement, positive.

at 37°C for 1 week, readjusting the pH from time to time to the original (The pH change occurred only within the first two days). Then after aerating off of toluene, it was neutralized with 1% HCl and diluted to 0.1% concentration of the substance with physiological saline and the filtrate was placed for 15 minutes in a boiling water-bath. The solution still gave the positive cancer reaction (S. Fig. 7).

B. Oxidation of the Group Carbohydrates with Periodic Acid and the Properties of the Products

Separation of the oxidation products

Of each of the group carbohydrates, 50 mg. were dissolved in 2 cc. of water, and after addition of 4 cc. of 0.25 *M* HIO_4, stood at 20°C in a dark place for 8 hours until the reaction ceased. The mixture was further stood together with 2 cc. of ethylene glycol for 30 minutes at the same temperature and after dilution to about 30 cc., dialysed (for 3 days) for eliminating the low molecular products. Next, it was filtered and distilled in vacuo to about 5 cc. and 10 volumes of an alcohol-ether mixture (1:1 by volume) were added. The deposit was centrifuged, washed with alcohol and dried in vacuo (over H_2SO_4). 40 mg. of a white powder were given from either of the cancer and mucosa carbohydrate. The products will be referred to as **C-Ch (ox.)** and **M-Ch (ox.)** respectively.

Chemical properties of the products

Qualitative tests. Results:

Assay	C-Ch (ox.)	M-Ch (ox.)
Sakaguchi	+	+
Hiyama	+	±
Molisch	±*	±*
Reaction with Ehrlich reagent (red coloration)		
After alkaline treatment	+	++
Without prior treatm.	+	−
Schiff (for aldehyde)	++	++

* A dark green ring occurred with an above-attaching, thin, pale red layer which disappeared soon.

Paper partition chromatography of the sugar components. 20 mg. each of the oxidation product of the carbohydrates were hydrolyzed as above and the hydrolysates were neutralized and condensed to about 0.6 cc. 0.03 cc. portions were sampled to chromatograph by the technique also same as above. See Fig. 8. It was revealed that glucosamine and chondrosamine remained unattacked for a great part at least, but almost all of galactose and fucose had been distintegrated.

Biological properties of the products

The serological (The writers again owed to Dr. T. Miki in the Institute of Forensic Medicine). Regarding the techniques, cf. above.

1) Inhibition to isohemoagglutination. They were devoid of this potency (Table V).

2) Hemolysis inhibition. This activity was also found almost demolished (Table VI).

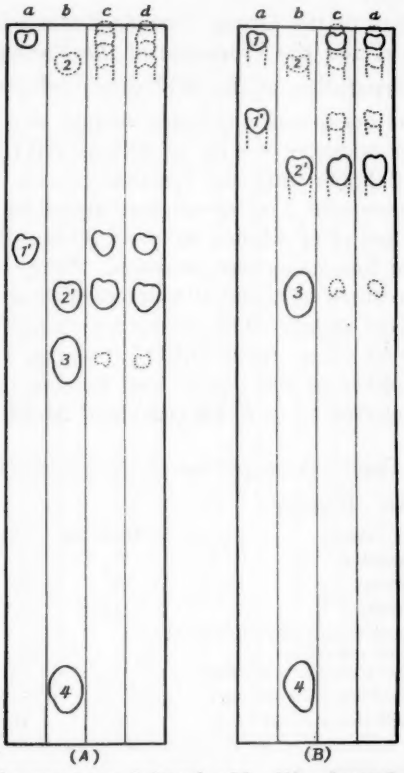


Fig. 8. Chromatograms irrigated with (A) *n*-butanol-pyridine-water (5:3:2) and with (B) *n*-butylacetate-acetic acid-ehtanol-water (3:2:1:1) of the hydrolysate of C-Ch (ox.) and M-Ch (ox.). Indicator, aniline hydrogen phthalate of Partridge. Temperature of test 22–26°C, duration of test 24 hrs. a, b: reference runs. c: C-Ch (ox.), d: M-Ch (ox.). 1, 1′: chondrosamine sulfate, 2, 2′: glucosamine sulfate, 3: galactose, 4: rhamnose.

TABLE V

Agglutination of Human Erythrocytes by Normal Sera treated with the Oxidized Cancer or Mucosa Group Carbohydrate

B and A serum had agglutinin titre 160 and were diluted 20-fold. ++: marked aggl.

Carbohydrate	Group of red cells	Dilution of substances (1:)			
		10^2	2×10^2	4×10^2	without substance
C-Ch (ox.)	A	++	++	++	++
	B	++	++	++	++
M-Ch (ox.)	A	++	++	++	++
	B	++	++	++	++

TABLE VI

Hemolysis by the Anti-A Rabbit Serum, treated with the Oxidized Cancer or Mucosa Group Carbohydrate, and Complement

−: no hemolysis, ±: faint hemolysis, +, ++ and #: partial hemolysis, ##: complete hemolysis.

Carbohydrate	Dilution of substances (1:)									
	2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3	6.4×10^3	1.28×10^4	2.42×10^4	without complement	without complement and substance
C-Ch (ox.)	±	+	++	++	++	##	##	##	−	−
M-Ch (ox.)	−	±	+	++	++	##	##	##	−	

Fig. 9

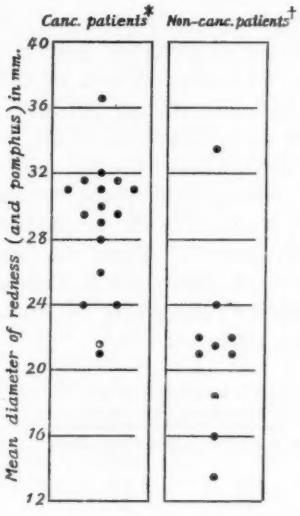


Fig. 10

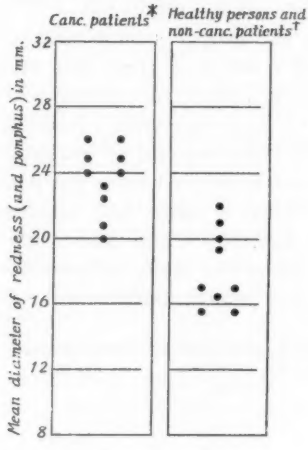


Fig. 9. Showing the skin reactions of C-Ch (ox.) (a 0.1% solution).
* carcinoma of stomach (7), oesophagus (3), colon (2), skin (1), liver (1), tongue (1) and external genitalia (1), and branchiogenous cancer (1).
† gastric (3) and duodenal (2) ulcer, hernia (1), appendicitis (1), fracture (1), intestinal stenosis (1) and bronchiolectasis (1).
Judgement, positive.

Fig. 10. Showing the skin reactions of C-Ch (ox.) reoxidized (a 0.1% solution).
*, † Individuals tested were the same as those in Fig. 7.
Judgement, positive.

Cancer skin reaction. Quite unexpectedly C-Ch (ox.) gave the positive cancer skin reaction (Fig. 9). Hence the treatment with HIO_4 was repeated to complete the oxidation: 15 mg. of C-Ch (ox.) were dissolved in 1 cc. of water by heating, and when cold, mixed with 2 cc. of 0.25 *M* HIO_4. The mixture was maintained at 20°C (in a dark room) for 45 hours. Fourtheron the remaining processes noted were followed and 8 mg. of a white Molisch-negative powder were given. It was active, too (Fig. 10).

Discussion

1. Calculated from the iodine values in Table I, the minimum molecular weight of the cancer and the mucosa substance isolated amount to 5600 and 3000 respectively, and when their amino acid grouping and ash are taken into account, the proper carbohydrate moieties (approximately 82 and 85% each to each) approach to m. w. 4600 and 2600 respectively, corresponding to molecules made up of about 13 and 7 disaccharide units. If a straight chain of recurring disaccharide units is assumed for both the proper carbohydrates, the non-reducing end of the chains consumes 2 moles of HIO_4 per mole, but the reducing (an acetylhexosamine) 1 or 2 moles according whether acetylhexosamines are bound at their 4- or 3-position with 1-position of the neighbouring non-amino sugars (That the reducing end of the proper carbohydrate chains are an acetylhexosamine is derived from the positive reaction of the preparations with Ehrlich reagent after hot alkaline treatment). Since the substances reduced 3.32×10^{-3} and 3.15×10^{-3} mole of HIO_4 per g., namely 18.6 and 9.5 moles per mole, moles of the oxidant reduced by the intermediate part of the proper carbohydrate chains come to:

HIO_4 consumed by the terminals (moles)	Group carbohydrate	HIO_4 assignable to the intermediate part (moles)	
		as a whole	per disaccharide unit
3	C-Ch	15.6	1.3
	M-Ch	6.5	1.1
4	C-Ch	14.6	1.2
	M-Ch	5.5	0.9

In both of the group carbohydrates, non-amino sugars must be combined by the adjacent hexosamine glucosidically at their 2- or 4-position, because, first, all the non-amino sugars were found oxidized, contrary to the amino sugars, and, secondly, the HIO_4 uses by the respective intermediate parts per disaccharide unit are not far remote from 1 mole regardless of the amount of the oxidant reduced by the terminals. The amino sugars (N-acetylated) were not oxidized, probably the one standing at

the reducing terminal alone excluded. Hence their 3- or 4-C are regarded as bound by 1-C of the adjacent non-amino sugar.

As mentioned above, the preparation from the mucosa was a product of the native substance partially cleft during preparation. The intact molecule of both of the group substances may contain two shorter proper corbohydrate chains bound at the non-reducing end of one chain and the reducing of the other through an alkali-labile link, group or groups in such a way as does not interfere with the periodic acid-oxidation. If so, it is easily elucidated that, in the preparation from the cancers, the intermediate part of the joined long proper carbohydrate chain used somewhat more than 1 mole of HIO_4 per disaccharide unit, since the reducing end of the second short chain, which is analogized to be a hexosamine from above, can reduce 1 mole or more of HIO_4, notwithstanding that it is localized in the intermediate part of the long chain. Thus the structural identity might be assumed between the carbohydrate moiety of the two group substances in native state.

The preparations were pretty strongly levorotatory (Table I). Therefore, the links between neighbouring two hexose units are considered mostly β-glucosidic to say the least of it. Furthermore it looks probable that 4-C of hexosamines and 2-C of galactose and fucose are the positions actually partaking in the linkages, because a compact coil structure like the group carbohydrate from pig stomach mucus of Masamune *et al.*[9] can be formed by this mode of union.

2. The cancer reaction-giving structure in the group carbohydrate from the cancers was decomposed neither by tryptic digestion nor by periodic acid oxidation. It needs no saying that trypsin can only attack restricted kinds of peptide link. Hence the residue in question is not precluded to make a part of the peptide. The proper carbohydrate moiety does not appear like the possessor of the potency, which was still effective after the HIO_4-oxidation of the substance, contrary to the serological activities examined. Further data are requisite to settle the problem.

Summary

1. Group A carbohydrates were prepared from gastric cancers and human gastric mucosa, and their properties were investigated.

2. They resembled each other in qualitative and quantitative composition.

3. The structure of their proper-carbohydrate-moieties was discussed and close similarity between the group carbohydrates in this respect was suspected.

4. But significant biological discrepancies were shown between the

group carbohydrates. Explanation awaits further experiments and contemplation.

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